Azo Dyes as Ionochromic Anion Indicators and the Synthesis of 1,2-Diphenylethane-1,2-dione

Research Thesis

Presented in partial fulfillment of the requirements for graduation *with research distinction* in Chemistry in the undergraduate colleges of The Ohio State University

by

Sydney McKee

The Ohio State University

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Project Advisor: Dr. Noel M. Paul, Department of Chemistry and Biochemistry

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Chapter 1: Azo Dyes as Ionochromic Anion Indicators

Abstract

Organic azo dyes, which contain the nitrogen-nitrogen double-bonded azo functional group (-N=N-), have found wide use as valuable textile dyes, tissue stains, and pH indicators. These highly conjugated systems can be synthesized via the electrophilic substitution of activated aromatic rings with arenediazonium salts, resulting in molecules that exhibit high molar absorptivities within the visible light range. Originally, it was thought that structures based on 1,8-bis(dimethylamino)naphthalene may have led to dyes that could exhibit ionochromic sensitivity to various aqueous anions. However, it was found that potential dyes containing this motif were impure and difficult to isolate. This structure was thus abandoned in favor of coupling the diazonium salts with a wide variety of nucleophiles in both aqueous and organic media in order to find an optimal synthetic strategy. Particularly pure dyes were exposed to varying pH and anion concentrations in a range of solvents, and the changes of their UV-vis spectra were assessed. These experiments have provided guidance for the identification of future candidates for further analysis. In the context of water sanitation and health, a successful indicator would serve as a facile and cost-effective method of identifying hazardous anion concentrations in drinking water.

1.1 The prevalence of anions and the necessity for their screening

Currently, more than one billion people across the world lack access to clean drinking water.[i] Therefore, the purification of water is imperative to modernized society; but first, sufficient detection methods for screening contaminants need to be employed or developed for subsequent proper treatment. Thus, the detection of potentially hazardous anions is critical to the sanitation of public drinking water, not only in the United States, but across the world. A variety of hazardous contaminants, such as anions, are present in the water and a myriad of detection methods have been developed in order to mitigate this issue.

Certain anions can pose detrimental health effects; for instance, the increasing presence of fluoride in drinking water and its excess ingestion can risk the development of fluorosis, which can lead to skeletal and dental damages.[i] Impairment of muscles can ensue if the ingestion of fluoride is chronic and of high dose through the calcification of ligaments and modification of bone structure, but in moderate cases, chronic ingestion can cause defects in enamel and teeth staining. Although fluoride ingestion can be from a myriad of sources, such as food, toothpaste, and even the air, the most common source of intake is from drinking water. Therefore, it is imperative to develop simple detection methods for fluoride to screen potentially affected water sources. Mild cases of fluorosis affect millions of people around the world, and so, proper screening of affected areas and the subsequent water treatment of contaminated sources can yield general health benefits.[i]

Coumarin motifs have been shown to screen for the presence of fluoride anions and salts in water and acetonitrile (Figure 1).[ii] As aforementioned, it is necessary to develop facile methods for the screening of this anion as a means to treat contaminated water to prevent the onset of fluorosis. These coumarin derivatives pose an efficient, simple way to determine quantitatively the amount of this anion. Interaction of fluoride with the indicator yields a silyl deprotection, which in turn causes the now deprotected indicator to fluorescence.

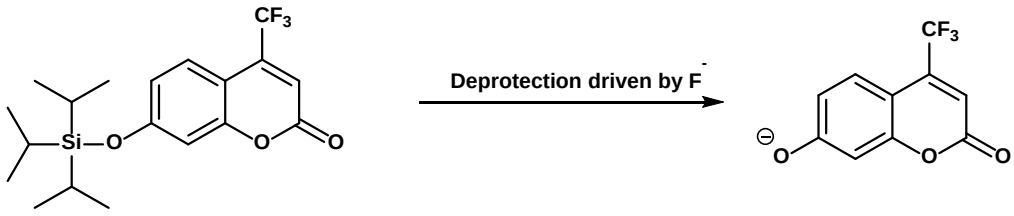


Figure 1. Coumarin derivatives fluoresce as a consequence of deprotection by the fluoride anion.

Thus, there is an interest in developing molecules, similar to the coumarin derivatives, that will yield an efficient, inexpensive method for qualitative appraisal of a sample. Molecules similar to this demonstrate the capacity for such a screening method to exist; therefore, azo dyes can fulfill a similar role on the basis of UV-Vis experimentation.

Another halide, bromide, can also pose health risks with unregulated exposure. A generally utilized fumigant, methyl bromide, has been correlated with prostate cancer in addition to psychomotor impairment as a consequence of chronic exposure.[iii] Methyl bromide has not shown appreciable impact on neurobehavioral performance, unlike sulfuryl fluoride, another harmful chemical that workers can be exposed to. Anions other than halides are also associated with detrimental health effects. For instance, nitrate is a common groundwater contaminant that originates from septic systems and fertilizers.[iv] Nitrogen in a fertilizer that is not utilized by plants can subsequently contaminate the environment as a nitrate. Infantile short-term exposure can lead to methemoglobinemia in the presence of bacteria, which corresponds to insufficient oxygen and potential death as a result. Although most intake of nitrates comes from a typical diet of vegetables, consuming water above a safe nitrate threshold mandates an alternative source since there is not simple way to dispose of these anions in a home setting. Therefore, it is imperative to determine an efficient, simple method of screening for nitrates, among other anions, for subsequent treatment of the water to avoid health complications imposed by their consumption.

1.2 Conventional methods for species screening

A few commonly used methods exist, including ion-selective electrodes and ion chromatography, to facilitate anion identification. The ion-selective electrode is composed of a reference cell along with the selective electrode itself within the same vessel as a galvanic half-cell.[v] Additionally, an external reference electrode is submerged within a reference electrolyte to compose the other component of the cell. An emf-response, E, is measured through the measurement of the total electrical potential difference. The ion-selective electrode has a ubiquity factor in that its detection can span a wide range of analytes with a degree of selectivity. However, this apparatus is only that: selective. Ion-selective electrodes fail in that their detection is not limited to the exclusivity of a single ion. Rather, this measurement is prone to interference from other species. Namely, monovalent cations are especially prone to these interferences, which is not as significant in regard to this study's focus on anion detection, but shows proof of principle.[vi] Additionally, ion-selective electrodes are prone to other factors affecting the reproducibility and reliability of response, and concentrations regarding the activity values of ions in solution can also impede the collection of accurate data.

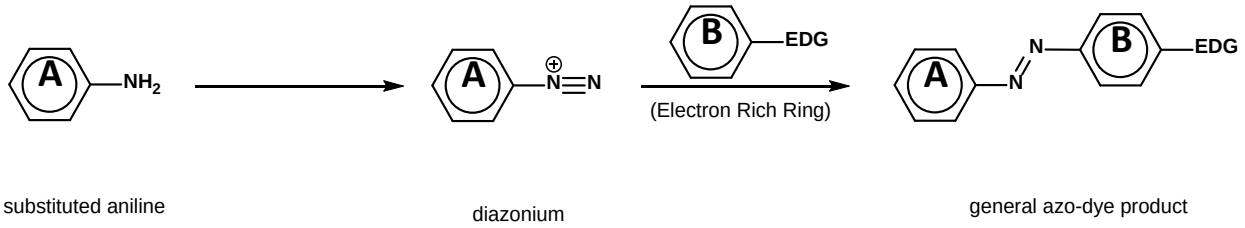
Ion chromatography instrumentation, such as high-performance liquid chromatography (HPLC) yields a viable method for the screening of different specific in a variety of media, including water. In fact, HPLC is widely utilized for the purpose of different environmental analyses, especially when there is a desire to deduce concentrations of species on a parts per million (ppm) or parts per billion (ppb) scale. Additionally, gas chromatograph can be similarly employed for the detection of hazardous species such as molecules composing herbicides.[vii] Comparing these methods between each other, there are advantages and disadvantages. Gas chromatography, when referring back to the herbicide detection example, mandates a much smaller use of sample and therefore can pose to be more sensitive than its HPLC counterpart. However, HPLC studies can yield more selective results than those obtained via gas chromatography, therefore harkening back to the similar issue ion-selective electrodes encountered in

their guarantee selectivity, but not exclusivity. Additionally, ion chromatography instruments are expensive and immobile, and sample preparation is required that is specific to the anion and the type of sample.[vii] Analyzing and comparing these aforementioned methods, including gas chromatography, HPLC, and ion-selective electrodes, show that all have their detriments with the most problematic issue being selectivity.

Thus, a selective anion indicator that is portable, cost-effective, and useable by non-experts would be of great value to the public health, and organic azo dyes could serve to fulfill all of these requirements. More so, the use of an azo dye as a colorimetric indicator is an efficient and simple method of identifying analytes in a sample that does not require complex instrumentation. Ideally, one could take the appropriate dyes on-site with a portable UV-Vis spectrophotometer and monitor spectrum behavior before and after the addition of the desired solution to be tested. This would address the immobile nature of ion chromatography instruments, and by utilizing a library of different azo dyes contingent on the anion of interest to be screened, this method can potentially bypass the exclusivity problem ion-selective electrodes and gas chromatography instruments encounter.

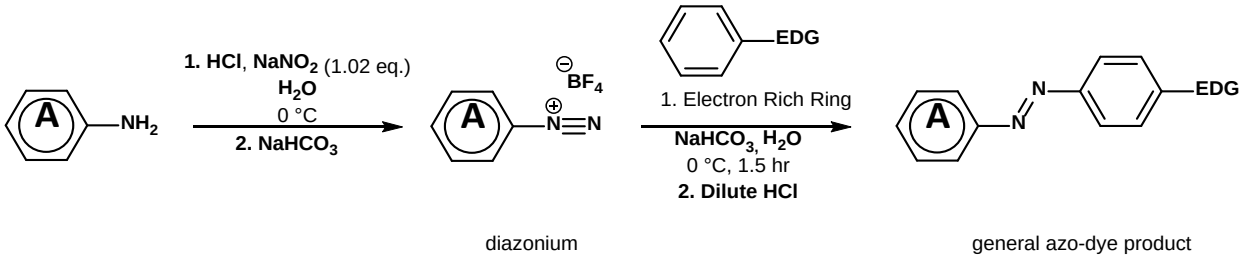
1.3 Methodology for the synthesis of azo dyes

The creation of diverse azo compounds is complicated by the inherent instability of the aromatic diazonium ions in solution that are required for their synthesis, as well as the number of mechanistic pathways available in the presence of these reactive intermediates.[viii] Many synthetic routes to achieve these products exist, the most common being the electrophilic aromatic substitution (EAS) reaction of an arenediazonium ion on a nucleophilic (electron rich) aromatic ring in aqueous acidic solution to yield the aforementioned azo functional group (Scheme 1). Although azo coupling in the presence of unprotected primary and secondary amines has been reported, isolation of pure products using this methodology has been difficult to reproduce.[ix]



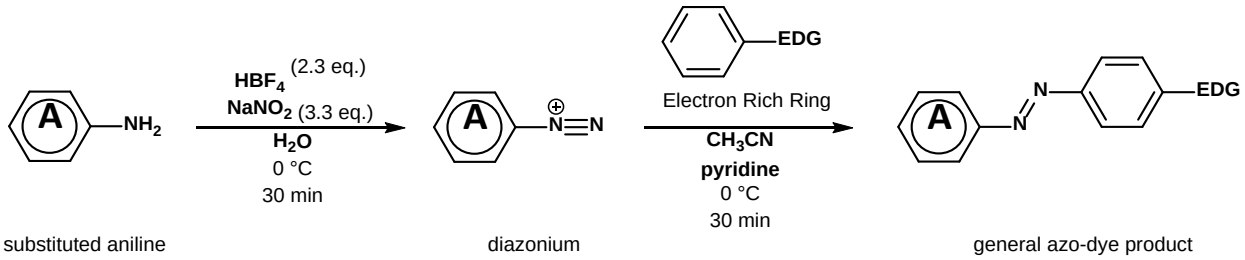
Scheme 1**.** General reaction scheme for the synthesis of azo dyes.

The typical "one pot" method of azo dye synthesis includes the formation of the diazonium salt in acidic media with immediate coupling to a nucleophile (Scheme 2).



Scheme 2. General reaction scheme for the synthesis of azo dyes utilizing the "one pot" method.

Based on previous work, however, the synthesis of diazonium tetrafluoroborate salts in organic media was now feasible. The benefit of this coupling strategy is that the salts can be synthesized, stored, and saved for later azo dye synthesis. This also allows for a broader context of synthetic strategy, as multiple simultaneous reactions with the same electrophilic salt can be prepared. This synthetic route additionally demonstrates the unique stability of aromatic diazonium salts, in which under normal circumstances would decompose due to their inherent instability. However, with proper storing conditions, these salts can be saved for extended periods of time. The methodology includes the formation of the arenediazonium salt in tetrafluoroboric acid, which can then be isolated for later usage, which includes the nucleophilic attack of an electron rich ring in organic media (Scheme 3).



Scheme 3**.** General reaction scheme for the synthesis of arenediazonium salts and subsequent synthesis of the general azo product.

This newly accessible method now allows for the generation of a wide library of azo dyes. A detriment previous indicators and screening methods encountered was an issue of exclusivity. However, with the conception of a broad azo dye library, there is the potential that this issue could be bypassed through the synthesis of specific dyes to exclusively screen for specific species.

1.4 The origins of colorimetric indicators from the REEL project at The Ohio State University

This project is a continuation of previous work with different iterations of the azo dye based on the simplification of Sudan Black B, a water insoluble lipid stain. Initial work involved the students of Chemistry 255/2550 in the REEL (Research Experience to Enhance Learning) project at The Ohio State University, and was continued by researchers who were recruited after the completion of the course. The project included the synthesis of a variety of azo dyes based on 1,8-naphthalenediamine, but because the products proved difficult to purify by crystallization, UV-Vis characterization in the presence of different anions was performed on the crude mixtures. Amazingly, the conditions that were purported to furnish one molecule in particular, (*E*)-3-((4,5-diaminonaphthalen-1-yl)diazenyl)benzoic acid (abbreviated 3-ABA-ND) was identified by multiple students as having a UV-Vis absorption that shifted in the presence of bromide, which was similar to the ionochromic effect behavior originally identified in Sudan Black B. With the use of preparative HPLC, several molecules from the 3-ABA-ND reaction mixture were isolated, analyzed by high-resolution mass spectrometry (HRMS), and studied by UV-vis for ionochromic effects, but the dyes were synthesized in yields too low (less than 1 mg) to perform structural characterization by NMR.[x]

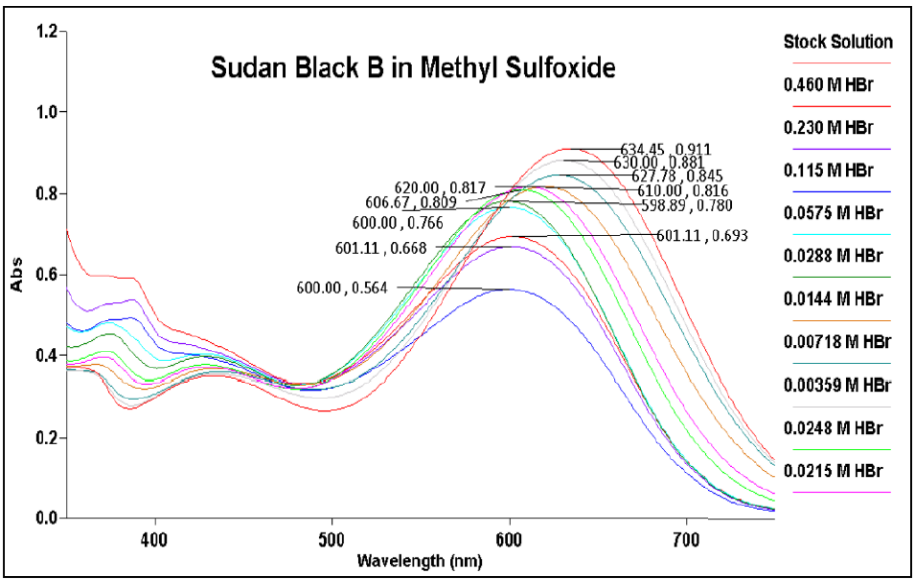


Figure 2. Bromide concentration vs. wavelength study of Sudan Black B in dimethyl sulfoxide (λ_{max} and A-value of each spectrum are noted). Unpublished results obtained by Heather Robison and are the property of the Ohio State University Department of Chemistry.

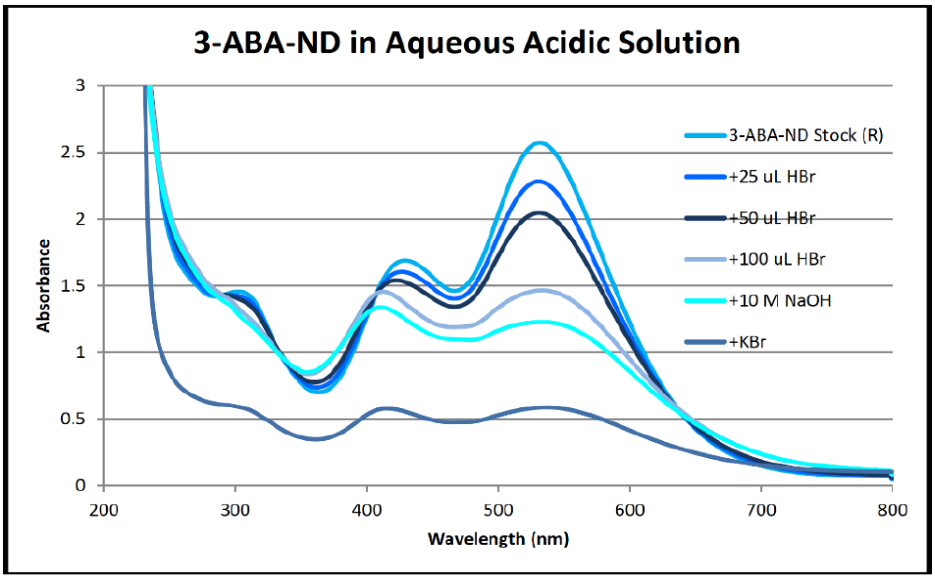


Figure 3. 3-ABA-ND vs. Bromide in Water. Unpublished results obtained by Heather Robison and are the property of the Ohio State University Department of Chemistry.

In a subsequent project, it was necessary to move beyond the typical water-based conditions for azo coupling, and methods to facilitate azo coupling using organic solvents under basic conditions were harnessed instead, as demonstrated by Scheme 3.[xi] With this advance, molecules that were once inaccessible were now feasible, including structures based on 1,8-Bis(dimethylamino)naphthalene, abbreviated TMND (Figure 4) and commonly referred to as "Proton Sponge".

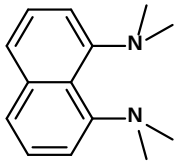


Figure 4. Structure of TMND, in an unrealistic protonation state.

1.4 Proton Sponge as a Nucleophile

Although initially utilizing the TMND motif was desired, subsequent attempted syntheses using this structure as the nucleophile yielded obstacles in regard to purification and isolation. The most prominent issue encountered with the actual coupling step arose on the basis of pH and complex acid/base equilibria. Each amine group possesses a lone pair, rendering them basic. In the presence of acidic media, either one amine has the potential to protonate, which greatly diminishes its nucleophilicity and therefore its ability to couple to an arenediazonium salt. This perhaps was not the issue with the coupling step, since it was carried out in basic media, but possible in the presence of acidic functionality on the electrophile. Initial synthesis attempted to couple 3-carboxybenzenediazonium tetrafluoroborate to Proton Sponge to yield (*E*)-3-((4,5-bis(dimethylamino)naphthalen-1-yl)diazenyl)benzoic acid, (**1**, Figure 5) employing conditions given in Scheme 3.

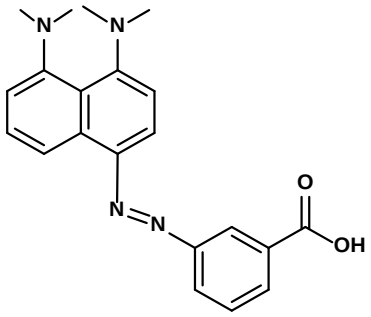


Figure 5. Initial synthesis attempt of **1**.

Following attempted syntheses of **1**, different isolation strategies were employed. Thin layer chromatography with 10% methanol in acetonitrile as the mobile phase revealed as many as four species composing the reaction mixture prior to purification. Solubility testing revealed isopropyl alcohol to be a sufficient recrystallization solvent; however, attempted recrystallization efforts failed. An alternative strategy was to employ preparative thin layer chromatography, using 10% methanol in acetonitrile as the mobile phase. The crude dye was dissolved in hot methanol and elution revealed five distinctly colored bands. Isolation of these bands and subsequent proton NMR in deuterated acetonitrile revealed incomplete synthesis and massive impurities. Following this discovery, different electrophiles were coupled with proton sponge in an attempt to overcome the complex acid/base equilibria that was potentially present, especially in the existence of the ionizable carboxylic acid on the 3-ABA salt (Figure 6).

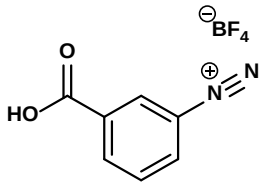


Figure 6. 3-ABA diazonium tetrafluoroborate salt for initial azo syntheses.

The subsequent electrophile of interest used 3-fluoroaniline as a precursor. Attempted coupling with Proton Sponge showed again multiple spots evident under UV light following the reaction using thin layer chromatography, implying an incomplete reaction, byproducts, or impurities. Recrystallization from distilled water was attempted but failed; proton NMR in deuterated acetonitrile revealed the same issues encountered with the previous coupling with the 3-ABA salt. An alternative purification strategy through extraction was then attempted. The crude dye was extracted using 1 M sodium hydroxide and methylene chloride, with the hope of deprotonating the basic nitrogen atoms evident in the structure to extract the dye into the organic phase. Subsequent thin layer chromatography results of the extracted layer utilizing 10% methanol in acetonitrile as the mobile phase showed multiple species under UV light, insinuating either the extraction had failed, or that the product was too impure to isolate efficiently.

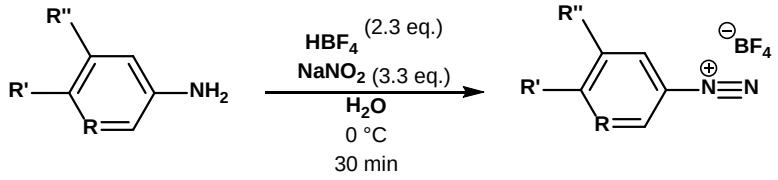
The next electrophile candidate screened was the tetrafluoroborate salt with 3-nitroaniline as the precursor. As with all previously attempted syntheses with different electrophiles, monitoring during and after the reaction via thin layer chromatography revealed multiple spots under UV light. As before, recrystallization from ethanol was attempted, but no crystals were evident. Extraction was also attempted utilizing 1 M sodium hydroxide and methylene chloride. The organic phase was evaporated under reduced pressure and analyzed via proton NMR using deuterated acetonitrile as the solvent; multiple expected aromatic peaks were missing from the spectrum, which suggested that perhaps these could be found in the aqueous layer. The aqueous layer was then evaporated under reduced pressure, and a proton NMR spectrum for this layer in deuterated acetic acid was obtained due to the solid from this layer being insoluble in acetonitrile. The spectrum presented an odd array of peaks and still showed the absence of the intended aromatic peaks missing from the organic phases' spectrum.

The previously attempted syntheses utilizing Proton Sponge as a nucleophile encountered a wide spectrum of issues, namely regarding the subsequent isolation and purification of the intended dye. Regardless of the purification strategy employed, whether extraction, preparative thin layer

chromatography, or recrystallization, all methods failed to prove the dyes were isolable or if the reaction even went to completion. Therefore, it was shown that Proton Sponge was not an optimal nucleophile to carry out azo dye chemistry. This could have been due to its unique basicity as a consequence of its bis-dimethyl amino structure. Thus, subsequent coupling was carried out with a variety of different nucleophiles, discarding Proton Sponge as a potential candidate.

1.5 Synthesis of diazonium tetrafluoroborate salts

Typical azo dye synthesis is usually carried out in a "one pot" method in aqueous media. Diazotization of an electron poor amine and subsequent coupling with an electron rich nucleophile occurs in two separate steps in the same experiment. This allows for easy and efficient coupling. As aforementioned, it was found that arenediazonium salts can show unique stability in proper storing conditions. A variety of different tetrafluoroborate salts, all from *meta*- or *para*- substituted anilines precursors, were synthesized with the hope of constructing a library of electrophiles to be coupled with a variety of nucleophiles in order to create a large array of different azo dyes with the capability of screening for different anions (Scheme 4).



substituted aniline

diazonium

2: R=CH, R'=H, R''=F
3: R=CH, R'=OH, R''=H
4: R=CH, R'=H, R''=COOH
5: R=CH, R'=H, R''=Br
6: R=CH, R'=H, R''=NO_2
7: R=N, R'=H, R''=H
8: R=CH, R'=H, R''=Cl
9: R=CH, R'=CN, R''=H
10: R=CH, R'=H, R''=SO_3H
11: R=CH, R'=CF_3 R'=H
12: R=CCOOH, R'=H, R''=COOH

Scheme 4. Diazonium tetrafluoroborate salts synthesized and isolated.

All salts were synthesized in a similar manner in accordance to Scheme 4. Although sufficiently stable for synthesis and storage, these tetrafluoroborate salts could not be reliably characterized owing to their extreme reactivity upon dissolution. Therefore, characterization methods such as GC-MS or NMR were not feasible due to imminent decomposition. In fact, literature reports that only a fractional amount of certain arenediazonium salts have been characterized via NMR.[xii] Infrared spectroscopy was largely an unreliable method of characterization, as the azo functional group appeared to be extremely sensitive to shifting contingent on groups in proximity. Therefore, by the virtue of their synthesis and isolation alone was proof of principle that stable diazonium salts could be stored and utilized for later coupling.

1.6 Synthesis of azo dyes utilizing the two-step coupling process in organic media

The majority of attempted azo dye syntheses relied on the method as prescribed by Scheme 3. After first synthesizing the diazonium tetrafluoroborate salts as depicted in Scheme 4, these electrophiles were subsequently coupled with commercially available electron rich nucleophiles, with the exception of 1-napthyl acetate, which was synthesized in accordance to literature.[xiii] All reactions were monitored via thin layer chromatography utilizing 10% methanol in acetonitrile as the mobile phase. Similar to results obtained with Proton Sponge coupling reactions, multiple spots were visible under UV light throughout the course of the reaction. Therefore, different purification techniques were employed as well. For **18**, the product was attempted to be extracted in order to isolate the baseline spot envisioned under UV light using 1 M sodium hydroxide and methylene chloride (Table 1). The aqueous layer was acidified and re-extracted to thus yield two isolated organic layers that were evaporated under reduced pressure and re-observed using thin layer chromatography. Results determined that both layers retained much of their impurities as evident by multiple spots under UV light. The initial reaction was then extracted with water and methylene chloride, and proton and fluorine (no decoupling and composite pulse decoupling) NMR data obtained also showed major

impurities in the product. This characterization was determined to be a common trend with most of the intended dyes to be synthesized from this route. Either their thin layer chromatography results showed many spots under UV light, and preparative thin layer chromatography measures did not appreciably isolate the corresponding dye, or other techniques employed to purify the dyes were unsuccessful when analyzing the NMR data obtained.

The exception to this general trend was observed with **13** (**13**, Figure 7). Utilizing the same conditions as shown in Scheme 3, the resulting product was evaporated under reduced pressure to yield a product when observed using thin layer chromatography to have only three spots, when normal conditions previously for other dyes saw much more streaking and impurities evident. When reviewing the product through NMR analysis using deuterated acetonitrile as the solvent, it was shown with no further purification attempts that the dye was certainly synthesized. However, the dye was also present with a 1:1 mixture of pyridine/pyridinium. No further isolation steps were performed to further purify the dye.

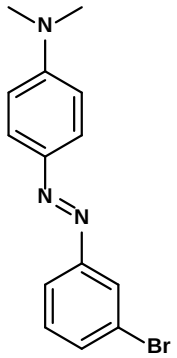


Figure 7. **13**, the only dye to be synthesized and isolated appreciably using the tetrafluoroborate salt method.

1.7 Synthesis of an azo dye utilizing the "one pot" method in aqueous media

Typical azo dye synthesis as taught in sophomore organic chemistry courses mandates the generation of an arenediazonium salt that is immediately coupled to a nucleophile all in aqueous media with mineral acid, thereby fulfilling the "one pot" approach. This synthetic strategy does not allow for the isolation of the generated electrophile, therefore these syntheses cannot capitalize on the ability to carry out many coupling reactions at a time with the same electrophile.

The successful reaction utilizing this approach was adapted from literature, following the general methodology as proposed by Scheme 2 to prepare **14** (**14**, Figure 8).[xiv]

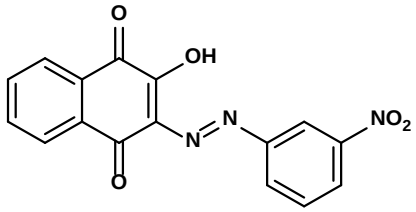


Figure 8. **14**, synthesized via both proposed methodologies.

Following the synthesis via the "one pot" approach, the product was attempted to be characterized via infrared spectroscopy. Peaks evident included the broad stretching near 3200 cm^{-1}, indicative of a hydroxyl group, the two nitro peaks close to the fingerprint region, and potentially the azo bond was evident but its stretching frequency appeared to be affected by the groups around it. Both methods attempted NMR characterization utilizing deuterated acetonitrile, but the product was insufficiently soluble. Solubility testing with methanol, acetonitrile, chloroform, water, and acetone revealed that acetone would be the suitable solvent to obtain NMR data. The spectrum obtained in this solvent showed the absence of impurities. Following this success, the product was also attempted to be synthesized via the tetrafluoroborate salt scheme.

Both the two-step method and the "one pot" synthesis were analyzed via thin layer chromatography utilizing pure acetonitrile as the mobile phase. The two-step coupling method, as with

earlier syntheses, showed many spots evident under UV light, whereas the one pot approach had far less streaking and spots prevalent. By also comparing with pure lawsone, a starting material, it was shown that the two-step synthesis showed a larger streak corresponding to lawsone than the one pot approach, which appeared to only have a residual amount when visualizing under UV light.

In order to further isolate the intended product from the two-step synthesis, a preparative thin layer chromatography plate was prepared by utilizing 25% acetonitrile in chloroform as the mobile phase and as the solvent to dissolve the dye. Following elution, five bands were visible by color and via UV light. The vibrant orange band evident on the plate, thought to be the intended product, was isolated and characterized via NMR with deuterated acetonitrile as the solvent. Upon observation of the spectra, it was determined that the reaction was either incomplete or riddled with many impurities. No further purification steps were attempted with this product.

Due to the success of lawsone as a substrate for the one pot approach to synthesize the intended azo dye, the next attempt was to see if the same success could be found by employing the same synthetic conditions but with juglone as a starting material, an isomer of lawsone. It was also of interest to determine if the juglone synthesis using the tetrafluoroborate salt approach would be isolable and pure, despite the synthesis for lawsone yielding no product with this approach. Following the synthesis of this dye (**15**, Figure 9) using both methodologies, the products were characterized via thin layer chromatography utilizing acetonitrile as the solvent and mobile phase. An additional lane for pure juglone was included, and upon elution, it was determined that the synthesis for both methods was incomplete. Both product lanes featured a spot for juglone and light streaking, but no other spots were evident. This suggested that there was a failed reaction, and other components in the reaction mixture could have contributed to the streaking evident.

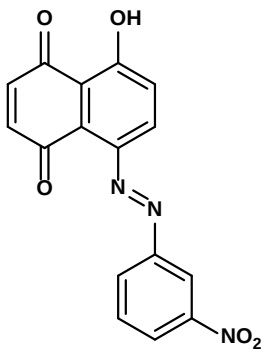
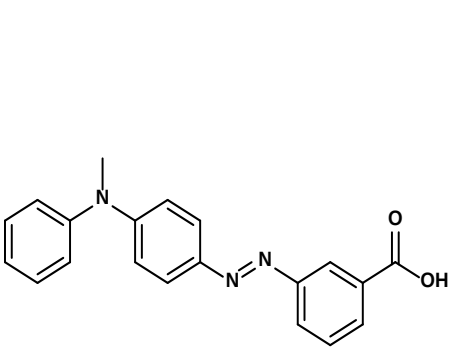


Figure 9. **15**, intended to be synthesized using both Schemes 2 and 3.

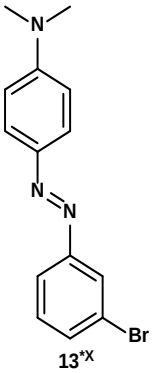
One of the proposed issues encountered with both the one pot method and the tetrafluoroborate salt method is controlling where the electrophile adds and how many times it adds. Ideally, the electrophile would add at the para position for most of the intended dyes and add only once. However, aniline derivatives are extremely activated substrates. Although it is probable that the first addition would be *para* due to steric hindrance evident at the *ortho* positions, there is potential that the electrophile could add at these positions too given a sufficiently activated substrate. This is one such reasoning that could explain the difficult to interpret NMR data for most of the intended syntheses. The other presumed issue regarding synthesis is the complex acid/base equilibria that nitrogen-containing molecules can exhibit. The nucleophilic anilines can reasonably be protonated under acidic conditions, rendering their ability to couple to electrophiles not as efficient or favored. However, the tetrafluoroborate salt method bypassed this issue with the coupling step by employing basic conditions in pyridine. This would hopefully negate this potential obstacle. The only attempted “one pot” approaches in mineral acid for both the generation of the electrophile and the subsequent coupling utilized non-nitrogen containing molecules, therefore maintaining the nucleophilicity. Lastly, an impurity evident for many of the two-step syntheses was the prevalence of pyridine/pyridinium in the reaction mixture. Extraction attempts and evaporating under reduced pressure did not appreciably mitigate this issue. At present time, it is unknown the general, pervading reason as to why many of the attempted

syntheses employed were riddled with impurities or failed to go to completion. It could be a combination of aforementioned issues, or due to entirely unrelated reasoning. The full attempted library of synthesized azo dyes can be found in Table 1. The discrepancy between the juglone derivative reactivity and the lawsone reactivity, and why the latter saw a successful synthesis utilizing the one pot method while the former had failed could be a consequence of juglone relying on the reactivity of the aromatic, phenol-like ring. This is in contrast with lawsone, which possesses reactivity more like an enol, which may have resulted in a slowed reaction.

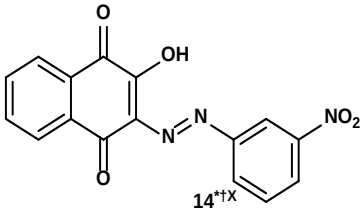
Table 1. Attempted synthesized azo dyes. Dyes denoted with * indicates Scheme 2, † indicates Scheme 3, and X indicates successful synthesis.



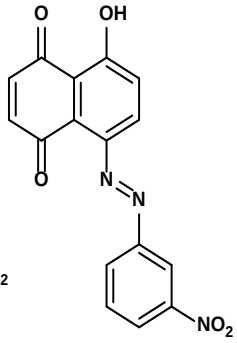
1*



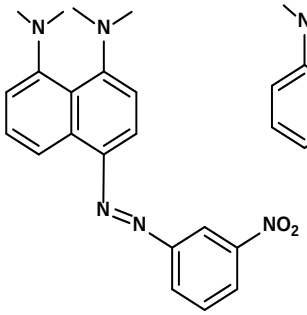
13*X



14*†X



15*,†



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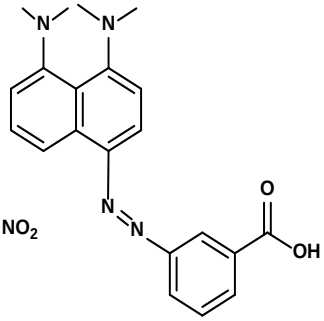

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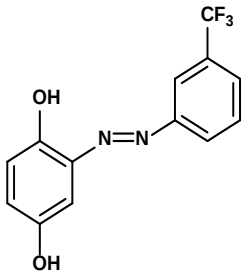

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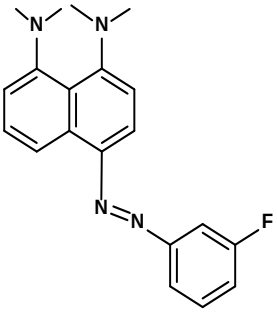

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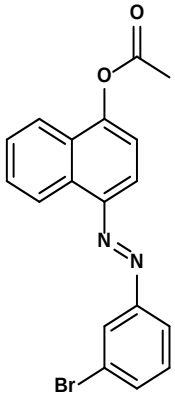

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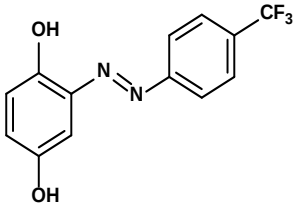

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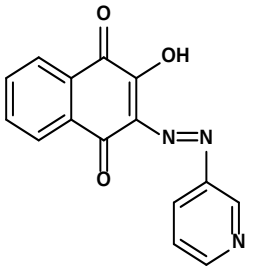


22*

1.8 UV-Vis studies of **14** in acetone

Initial UV-Vis studies had the addition of potassium bromide, potassium chloride, sodium nitrate, monopotassium phosphate, and dipotassium phosphate for **14** in acetone. However, these salts were not soluble in the testing solvents to an appreciable extent; as a result, the most concentrated solutions of the salt in 10 mM methanesulfonic acid in acetone that could be prepared were 0.2 M. In order to be consistent, the other soluble salts or acids were also diluted to 0.2 M initially. This concentration was deemed much too low that would allow for the screening of an observable effect on the dye's UV-Vis behavior for subsequent testing. For subsequent testing for **14** in acetonitrile and methanol, as well as for **13** in methanol, the aforementioned insoluble salts were discarded for the UV-Vis experiments in order to maintain consistency without compromising concentrated solutions. Instead, only the soluble salts and acids were utilized and their concentrations were prepared at 1 M in order to be able to screen evident changes in UV-Vis behavior of the dye for **14** in acetonitrile and acetonitrile and **13** in methanol.

Since **14** was soluble in acetone, this warranted the UV-Vis investigation among different anions. The stock dye solution of **14** in 10 mM methane sulfonic acid in acetone yielded a maximum absorbance at λ = 417 nm (Figure 10).

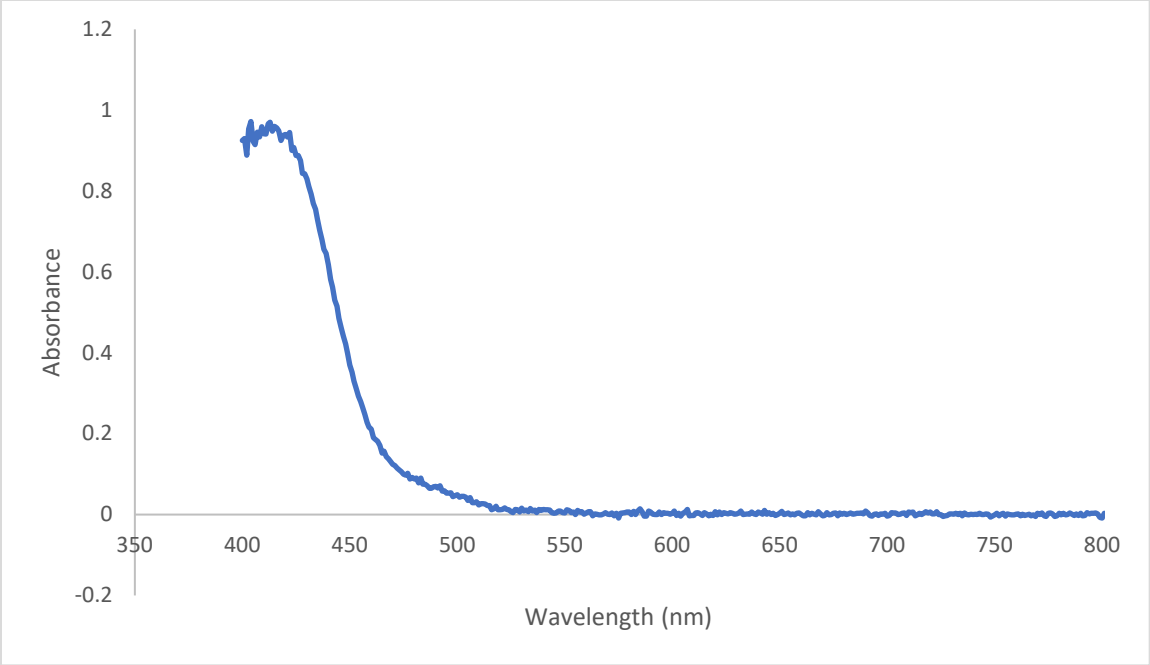


Figure 10. UV-Vis spectrum of **14** in 10 mM methanesulfonic acid in acetone.

Utilizing Beer's Law of A = εbc, the molar absorptivity of the dye at this wavelength was determined to be 1.58 x 10^4 cm^{-1} M^{-1}. Thus, the absorbance at this wavelength was monitored upon the addition of different anions to evaluate if new peaks became evident, or if there was a shift in its maximum wavelength. Any change in absorbance height at the initial maximum wavelength was irrelevant, especially if there was the absence of another peak increasing in absorbance simultaneously, due to the concentration of the dye decreasing with each increment of anion or base added. Upon addition of different anions at 0.2 M concentration in 0.1 mL increments, no observable effect other than an absorbance decrease due to dilution of the dye was evident. However, upon addition of 1 M NaOH in 0.1 mL increments, the solution remained the same color but white solid was evident in the volumetric flask independent of anion identity. Due to the presence of solid particles, the UV-Vis of the solution could not be accurately evaluated due to the scattering effects they would impose and thereby nullfiy Beer's Law. This warranted a new investigation to address the solid identity (Table 2). Interestingly, upon the addition of methanesulfonic acid, sodium hydroxide, chloroform, and **14** to a test

tube, no solid formation was evident. This insinuated that the presence of acetone was needed for the formation of the particulate.

Table 1. Preliminary testing of **14** to determine combinations yielding an insoluble salt.

Test Tube	**Species Added**	**Results**
Acetone	Sodium hydroxide	No reaction
14 in acetone	Sodium hydroxide	Dark orange solution
Methanesulfonic acid	Sodium hydroxide	Vigorous bubbling, no solid
14 in chloroform	Methanesulfonic acid	Yellow/orange solution
14 in acetone, methane sulfonic acid	Sodium hydroxide	Yellow solution initially; after addition of base, white solid evident
14 in acetone, sodium hydroxide	Methanesulfonic acid	Solution was initially orange and changed to yellow with white solid after acid addition
Acetone in sodium hydroxide	Methanesulfonic acid	Vigorous bubbling, no solid
14 in chloroform, methane sulfonic acid	Sodium hydroxide	No solid, but two layers evident (dark orange upper layer, light orange lower layer)

Due to this presence of solid, the capacity for the dye to function as an indicator in acetone was nullified as it would not be suitable for UV-Vis detection. The presence of the solid initiated the modification of future screening by adding the base in smaller increments and at a lower concentration. Subsequent testing was then determined to use 0.5 M sodium hydroxide, adding in 1 drop increments,

in order to titrate the pH back more slowly. However, even the addition of 1 drop of the diluted base showed the presence of solid. This further showed this dye's incapability of functioning as a sufficient indicator.

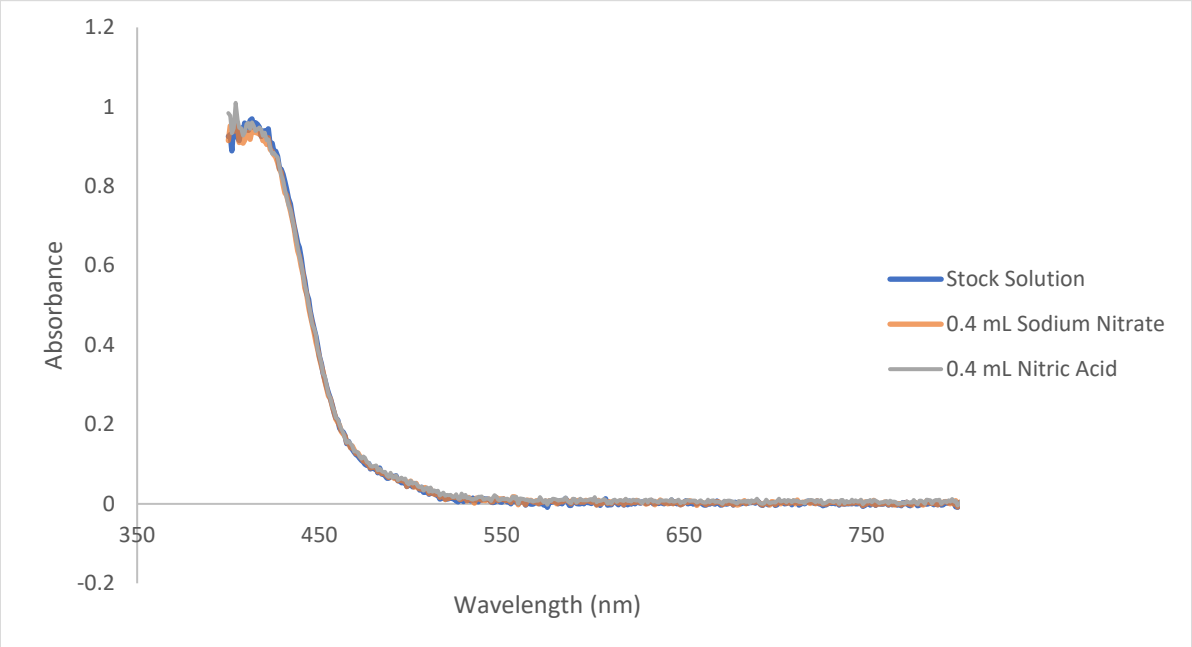


Figure 11. **14** in the presence of nitric acid, sodium nitrate. Addition of anion source did not impact the dye's absorbance.

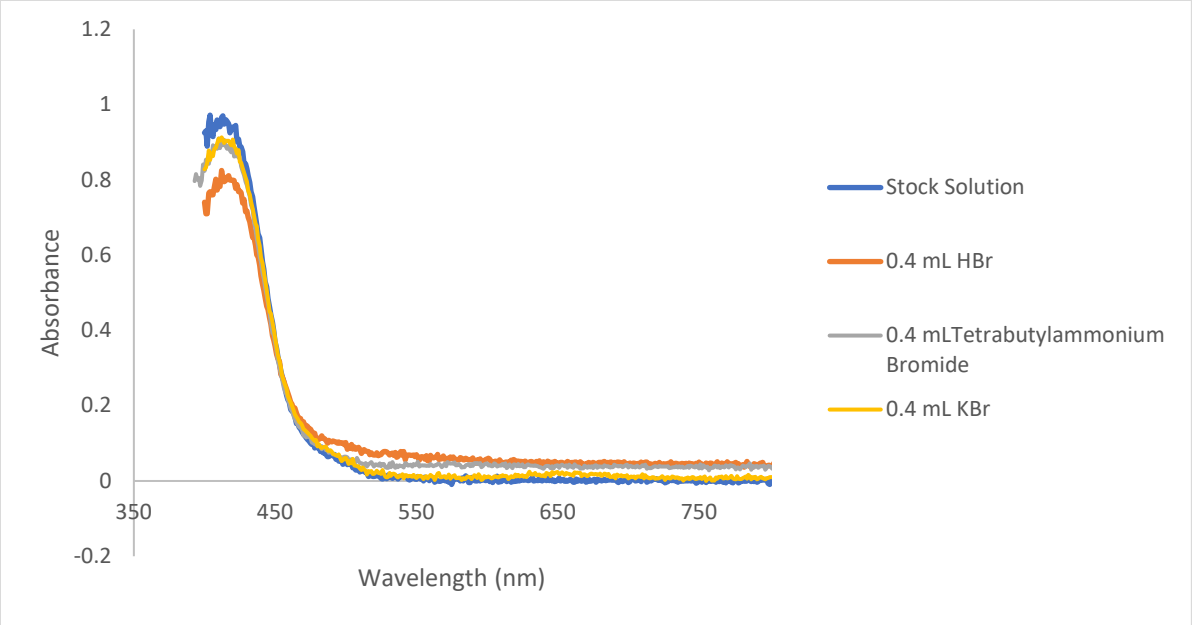


Figure 12. **14** in the presence of tetrabutylammonium bromide, hydrobromic acid, and potassium bromide. Addition of anion source did not impact the dye's absorbance.

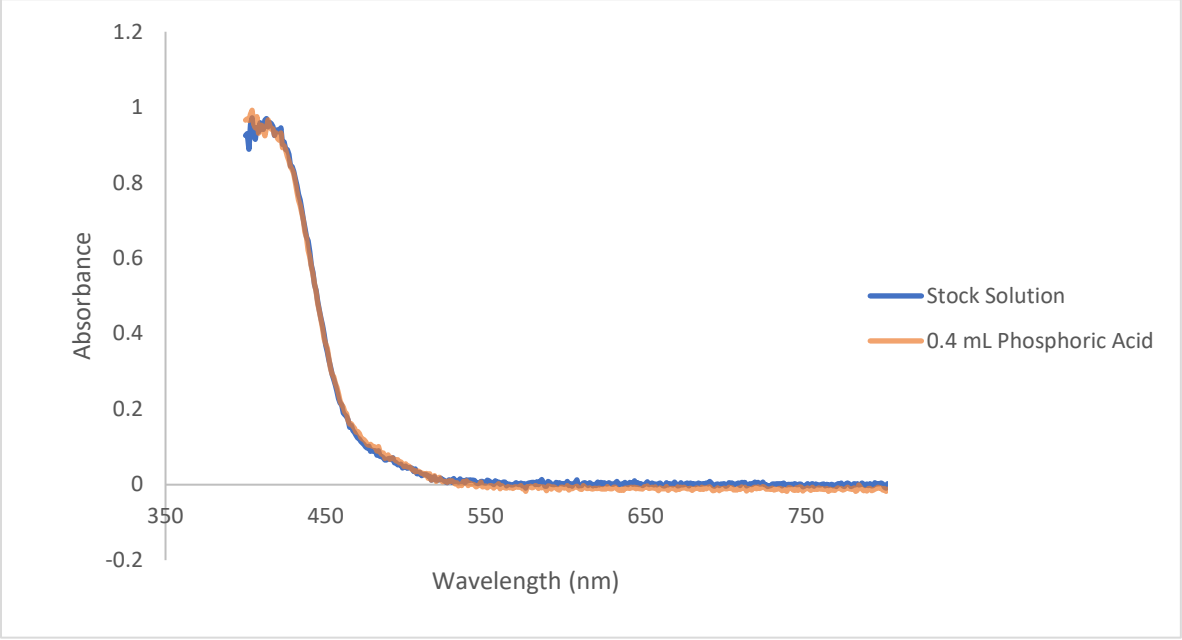


Figure 13. **14** in the presence of phosphoric acid. Addition of anion source did not impact the dye's absorbance.

1.9 UV-Vis studies of **14** in acetonitrile

The appreciable solubility of **14** in acetonitrile warranted further investigation to probe its UV-Vis behavior in the presence of a variety of anions. Following the same general procedure as noted in the Experimental, a dye stock solution was prepared in 10 mM methanesulfonic acid in acetonitrile to yield an absorbance of 1 at its wavelength of maximum absorbance at 416 nm (Figure 14). Utilizing Beer's Law in a similar fashion as earlier, the molar absorptivity of the dye in this solvent was determined to be 1.08 x 10^4 cm^{-1} M^{-1} at 416 nm.

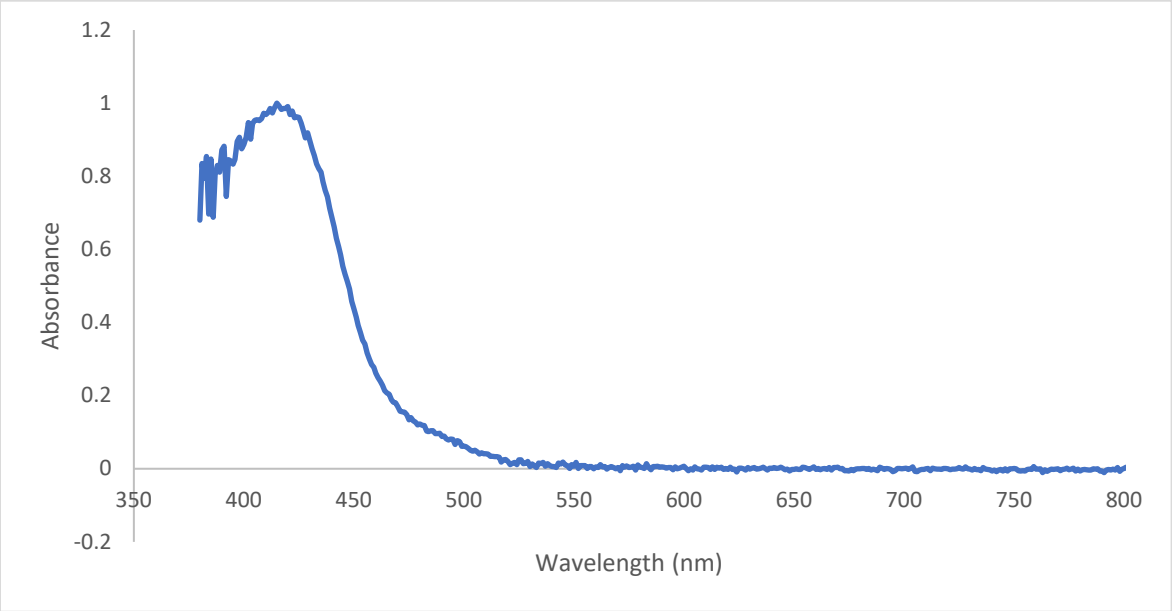


Figure 14. UV-Vis spectrum of **14** in 10 mM methanesulfonic acid in acetonitrile. Addition of anion source did not impact the dye's absorbance.

Anion solutions (1 M in 10 mM methanesulfonic acid in acetonitrile) were added in 0.1 mL increments to 10 mL of the dye stock solution to observe how its UV-Vis behavior changed. Tetrabutylammonium chloride was not appreciably soluble in the prepared solvent, and therefore it was omitted for this initial screening as well to keep all anion solution concentrations consistent. This solution's concentration was later adjusted to 0.2 M to include it in the study. Upon the addition of each anion solution, there were no apparent bathochromic or hypochromic shifts. Additionally, there did not

appear to be the prevalence of a new peak at the expense of a diminishing one as the increments of anions increased. The only observed effect was a decrease in absorbance at the dye's maximum wavelength, which was presumed to be due to the diluted concentration of the dye (Figure 15). After a total addition of 0.4 mL of each anion solution to separate 10 mL portions of the dye was completed, 0.5 M sodium hydroxide was added dropwise to note how pH would affect the dye's UV-Vis behavior. If there were a shift in behavior or the incidence of a new peak, then there would be a pH dependence for the dye's ability to function as an indicator. As noted previously, the acidic acetonitrile dye solution and 1 M sodium hydroxide saw evidence of a precipitate. For all anion solutions tested in acetonitrile, it appeared that solid particles were evident after a few drops of the sodium hydroxide solution was added. As such, UV-Vis data could not be collected for these solutions as the prevalence of solid particulate would nullify Beer's Law.

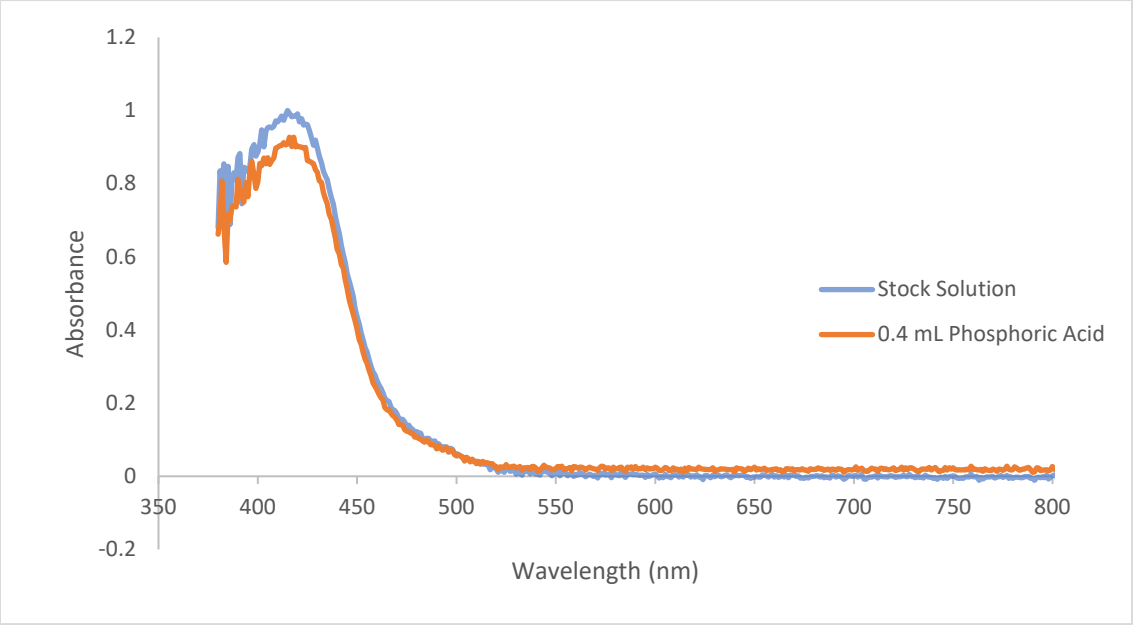


Figure 15. **14** in the presence of phosphoric acid. Addition of anion source did not impact the dye's absorbance.

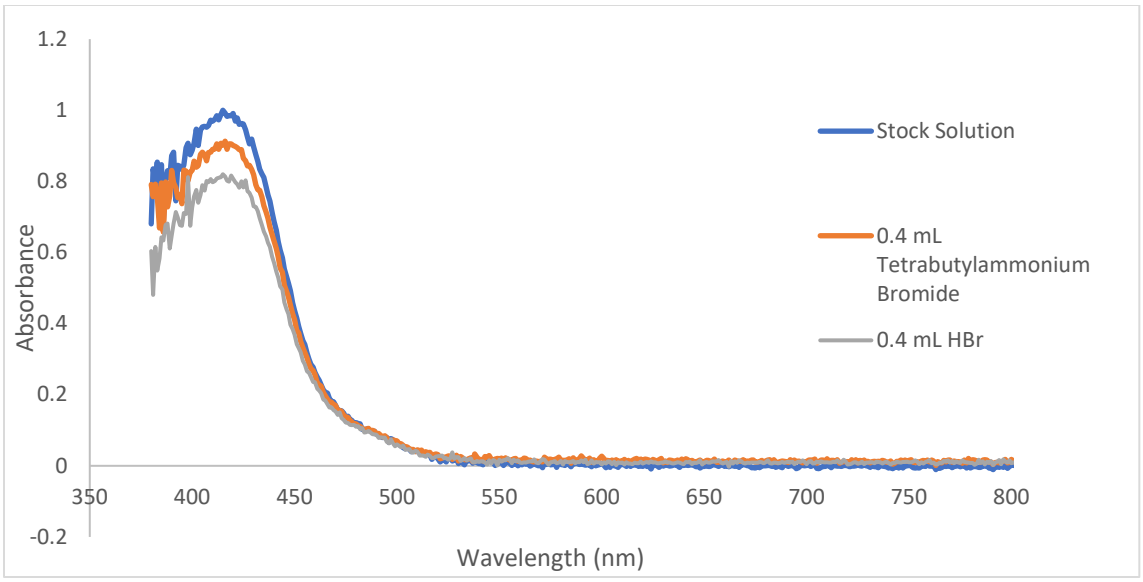


Figure 16. **14** in the presence of hydrobromic acid and tetrabutylammonium bromide. Addition of anion source did not impact the dye's absorbance.

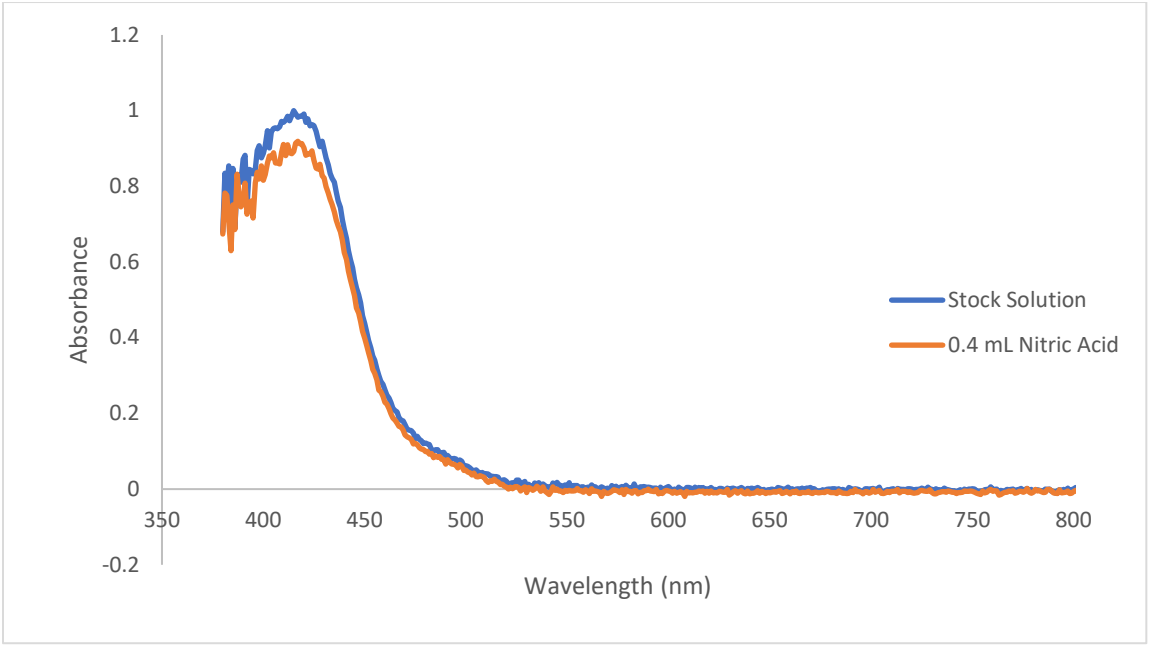


Figure 17. **14** in the presence of nitric acid. Addition of anion source did not impact the dye's absorbance.

1.10 UV-Vis studies of **14** in methanol

Lastly, **14** was analyzed in methanol employing a similar method in previous experiments. It was observed that regardless of anionic solution added and irrespective of concentration, no significant shift was observed in the UV-Vis spectrum for this dye. In regard to the stock solution, its wavelength of maximum absorbance was determined to be 413 nm, having a molar absorptivity of 1.32 x 10^4 cm^{-1} M^{-1} at this wavelength.

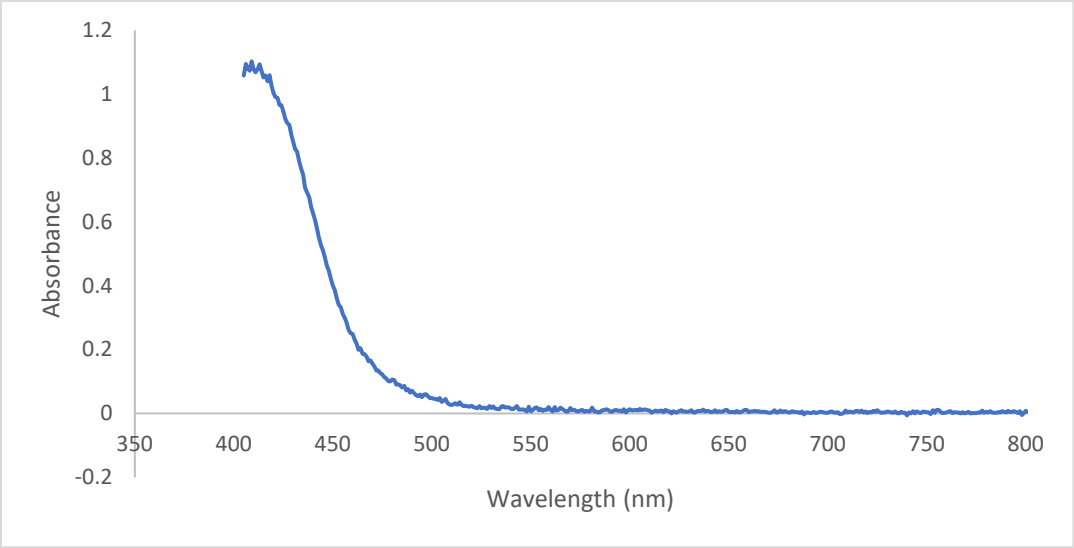


Figure 18. UV-Vis spectrum of **14** in 10 mM methanesulfonic acid in methanol.

Upon adding sodium hydroxide to the anion/dye solution, it was noted that no solid was evident for any anionic solution tested. This is in contrast with what was observed prior with the same dye in acetonitrile and acetone. This raised the question as to why the solid would be absent for the addition of base in this solvent; a proposed idea was that the product of potential reactants in the solution was appreciably soluble in methanol, but not in acetone and acetonitrile, which may validate this discrepancy. The incremental addition of sodium hydroxide did not impact the UV-Vis spectrum in any significant way. The absorbance of the largest peak diminished, but this was presumed to be a product of dilution of the initial stock solution and independent of any ion or pH effects.

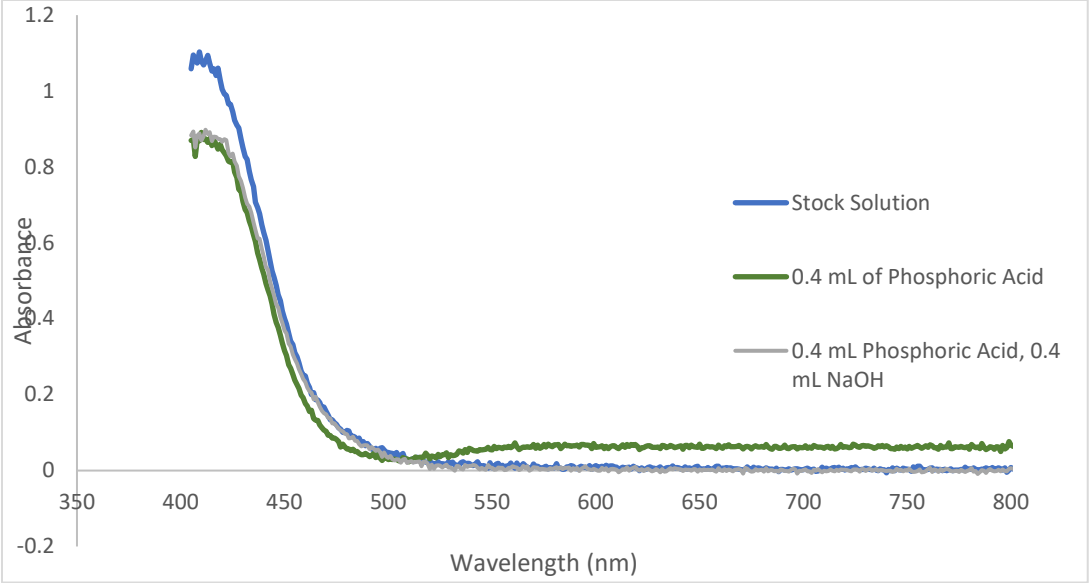


Figure 19. **14** in the presence of phosphoric acid and pH effects of added sodium hydroxide. Addition of anion source did not impact the dye's absorbance.

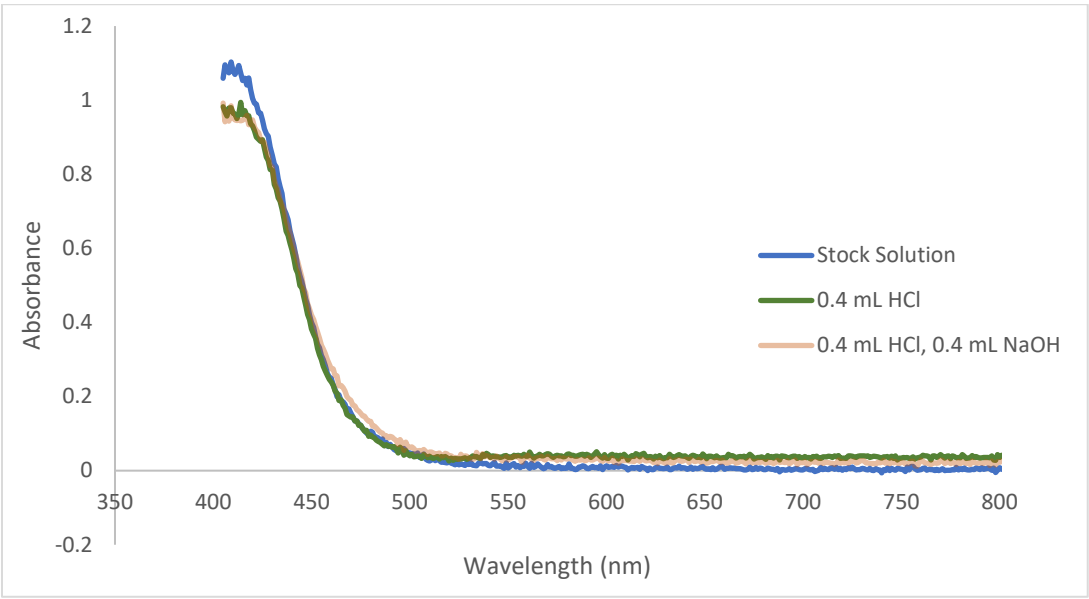


Figure 20. **14** in the presence of hydrochloric acid and pH effects of added sodium hydroxide. Addition of anion source did not impact the dye's absorbance.

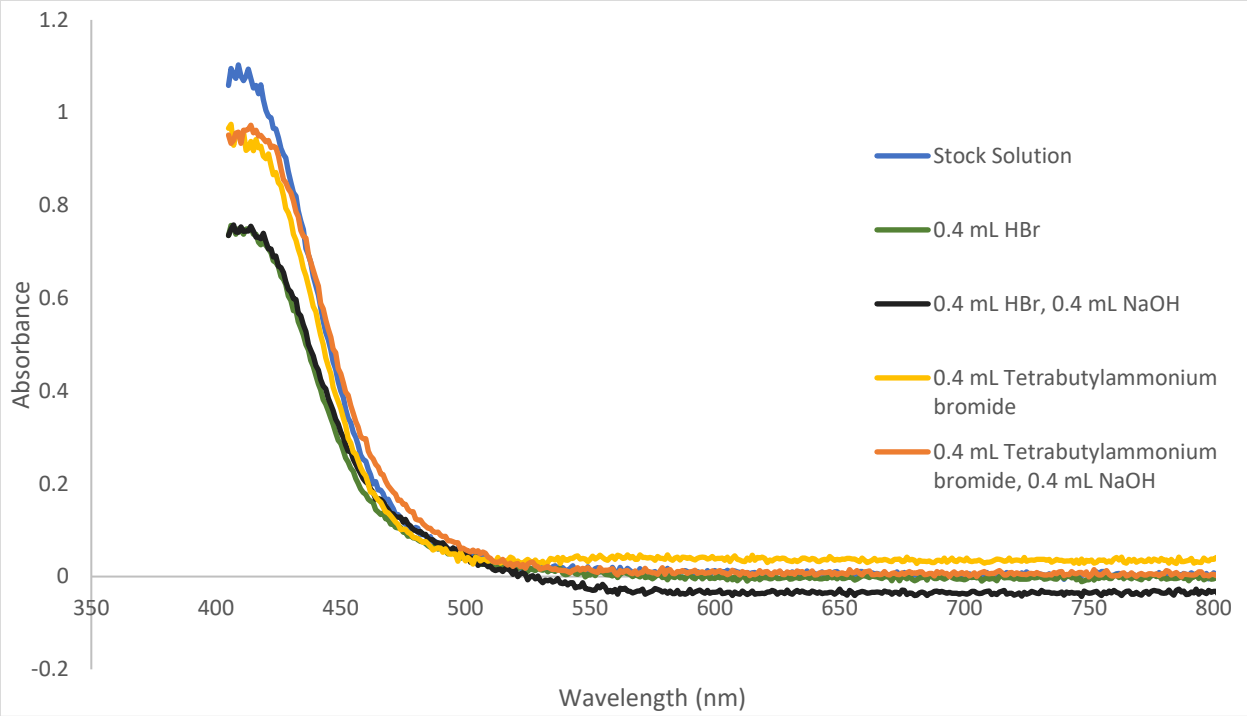


Figure 21. **14** in the presence of tetrabutylammonium bromide, hydrobromic acid and pH effects of added sodium hydroxide. Addition of anion source did not impact the dye's absorbance.

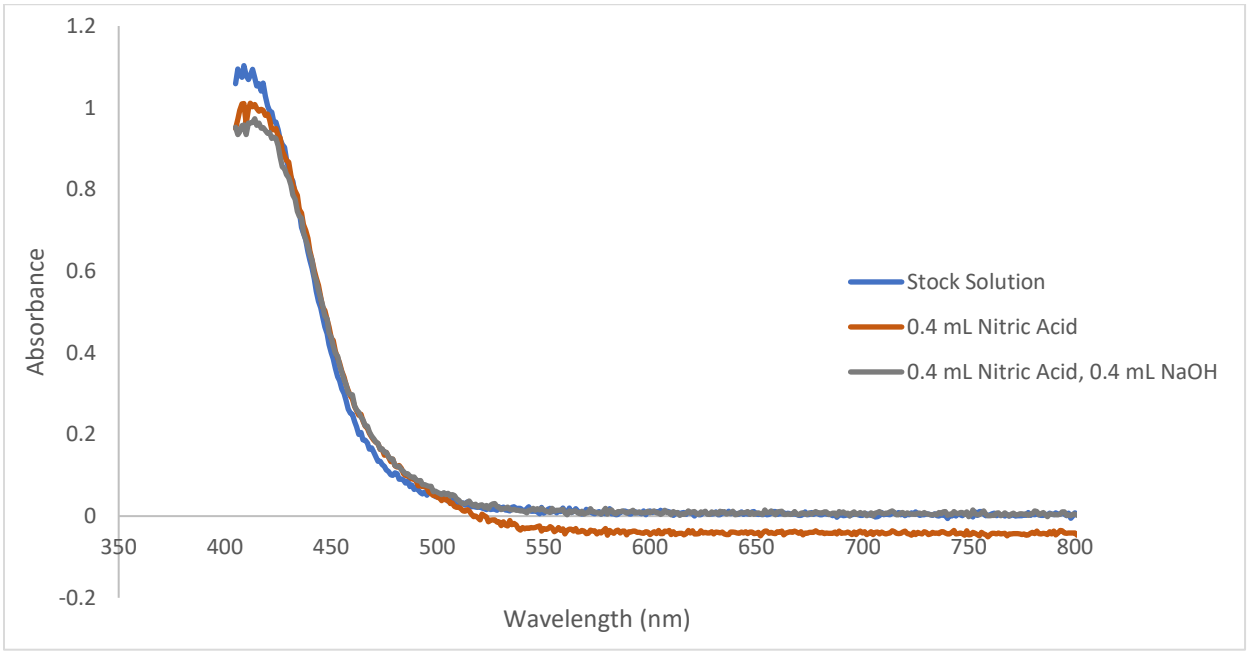


Figure 22. **14** in the presence of nitric acid and pH effects of added sodium hydroxide. Addition of anion source did not impact the dye's absorbance.

1.11 UV-Vis studies of **13**: methanol

Solubility testing of the isolated organic phase coupling dye warranted analysis in methanol only. As with previous stock solutions, dye was added slowly to a solution of 10 mM methanesulfonic acid in methanol until an absorbance of about 1 was obtained. Utilizing Beer's Law, it was determined that the molar absorptivity for the dye was 2.62 x 10^4 cm^{-1} M^{-1} at 508 nm (Figure 23).

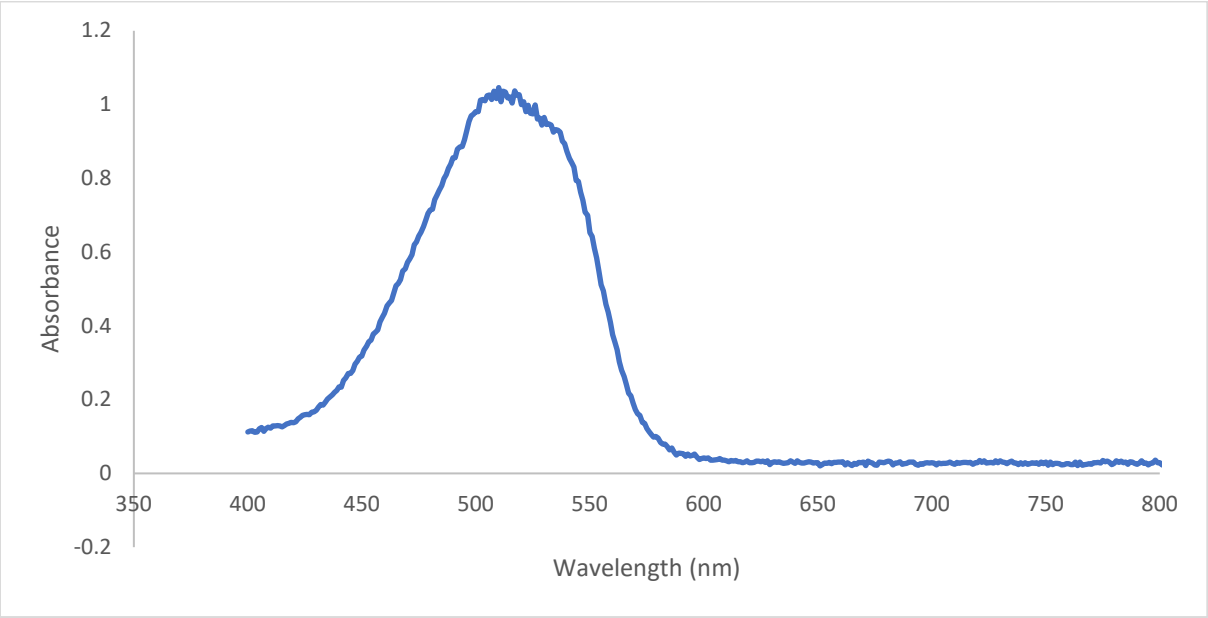


Figure 23. UV-Vis spectrum of **13** in 10 mM methane sulfonic acid in methanol.

As previously, 1 M anion solutions in 10 mM methanesulfonic acid in methanol were added to 10 mL of the dye stock solution in increments of 0.1 mL. As previously noted with other dyes, there was no significant shift for any anion at its highest concentration (a total of 0.4 mL added). In contrast with **14** in acetone, acetonitrile, and methanol, however, visible color changes and corresponding shifts in the spectrum were observed with the addition of sodium hydroxide in the tetrabutylammonium bromide solution. This was due to pH effects. The tetrabutylammonium bromide stock solution was only as acidic as the solvent it was dissolved in; that is, the 10 mM methanesulfonic acid in methanol, as opposed to the other anions added in solution which made the dye stock solution more acidic with each addition

(for instance, with phosphoric or nitric acid). Therefore, this anionic solution was much more sensitive the hydroxide being added. Therefore, with the change in protonation state brought on the change from pH, the conjugation of the dye changed which corresponded to a change in color of the dye solution (Figure 24). At pH = 1, the dye was a brilliant pink; with the addition of hydroxide to about pH = 5, the solution changed to orange, and with the further addition of base to an alkaline solution, the dye took on a vibrant yellow. On the basis that this dye was sensitive to pH effects due to the change of electronic density as a consequence of protonation state, demonstrates its poor ability to function as an anion indicator. A feasible dye would have UV-Vis spectrum behavior changes in the presence of different anions, and independent of pH.

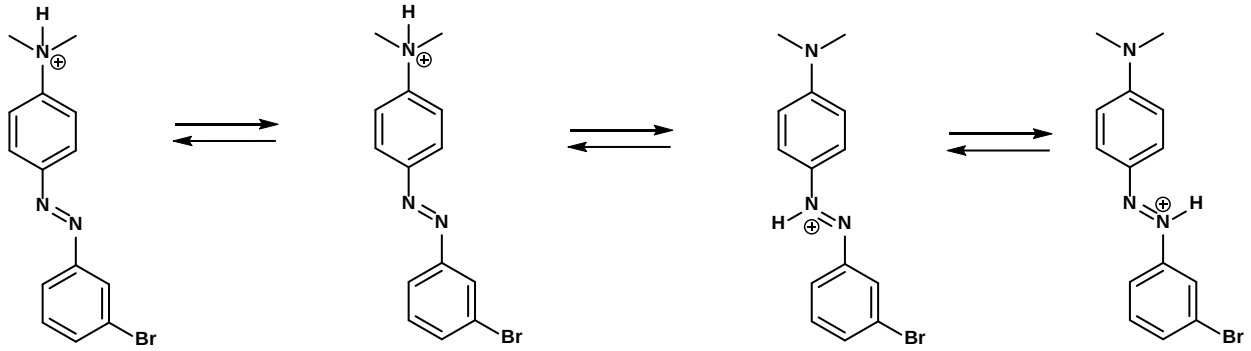


Figure 24. Potential protonation states of **13** explaining the change in color as a function of pH.

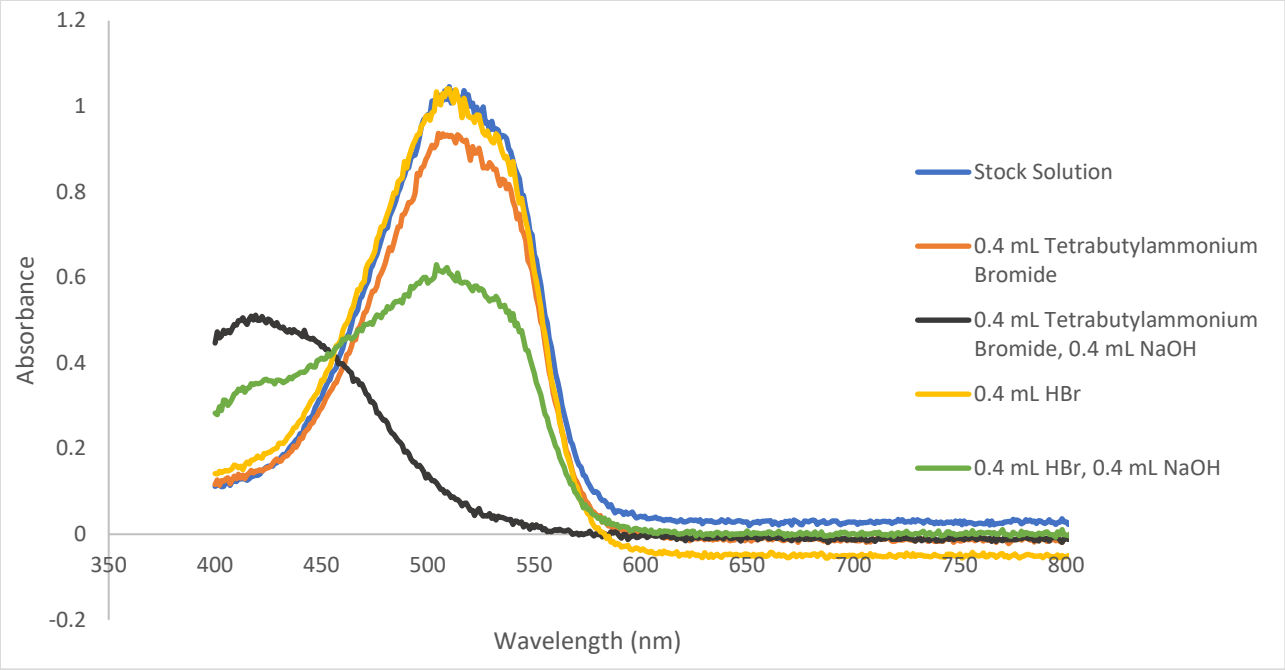


Figure 25. **13** in the presence of tetrabutylammonium bromide, hydrobromic acid and pH effects of added sodium hydroxide. Addition of anion source did not impact the dye's absorbance, but addition of base caused a hypsochromic shift for the less acidic anion solution tested.

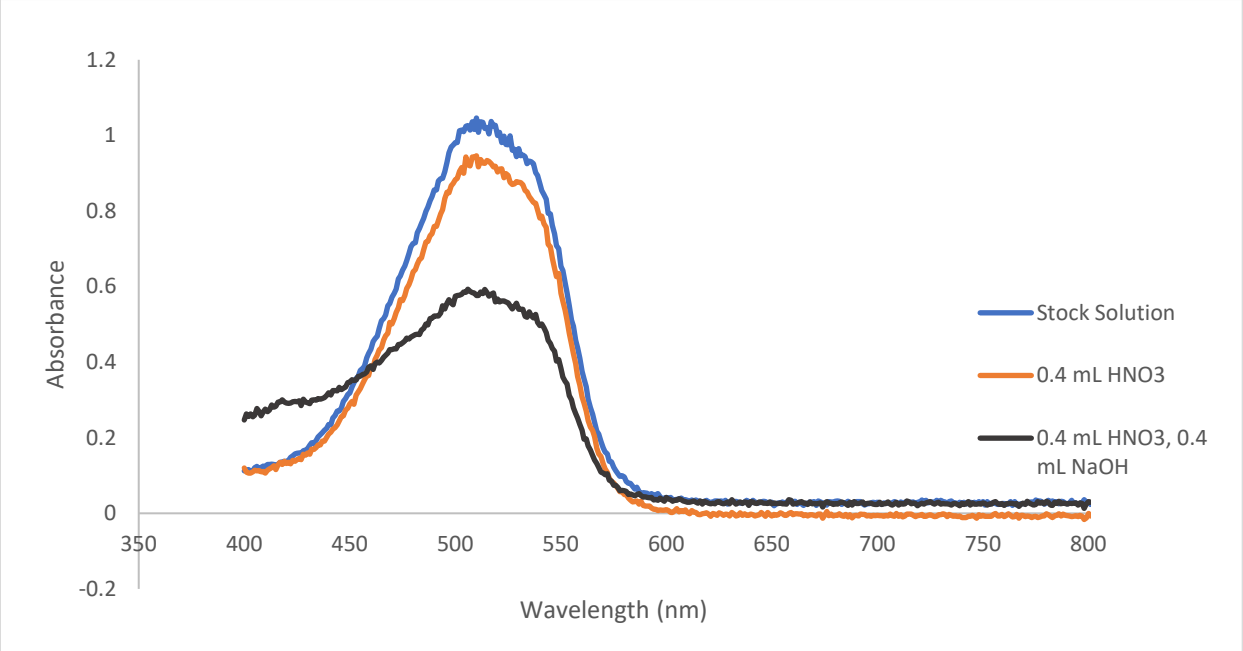


Figure 26. **13** in the presence of nitric acid and pH effects of added sodium hydroxide. Addition of anion source did not impact the dye's absorbance, but addition of base showed the addition of a shoulder.

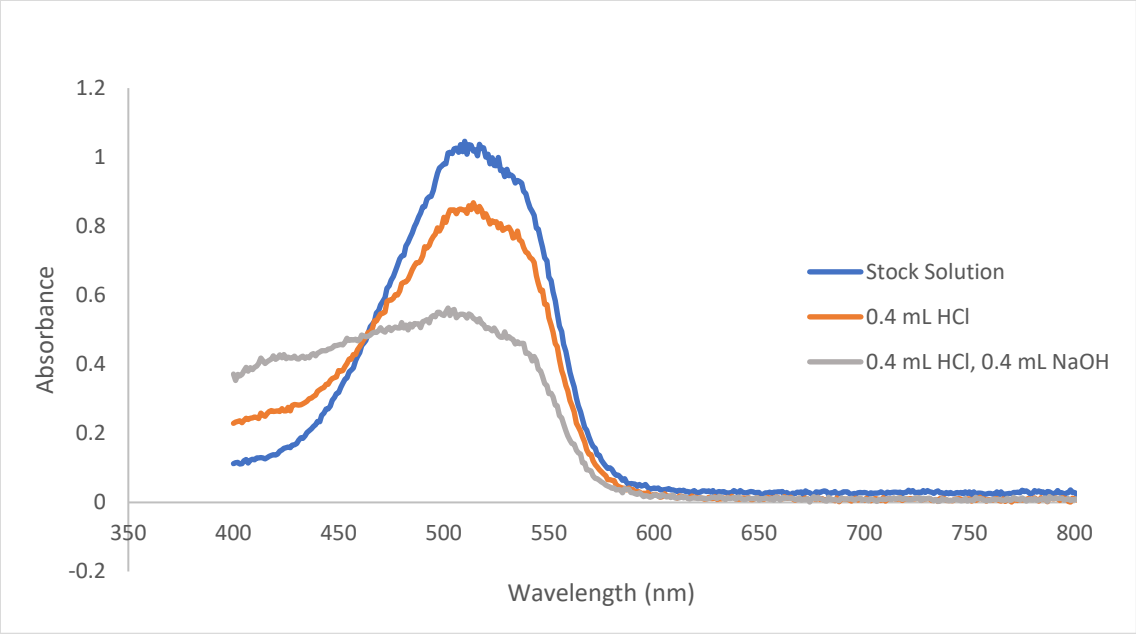


Figure 27. **13** in the presence of hydrochloric acid and pH effects of added sodium hydroxide. Addition of anion source did not impact the dye's absorbance.

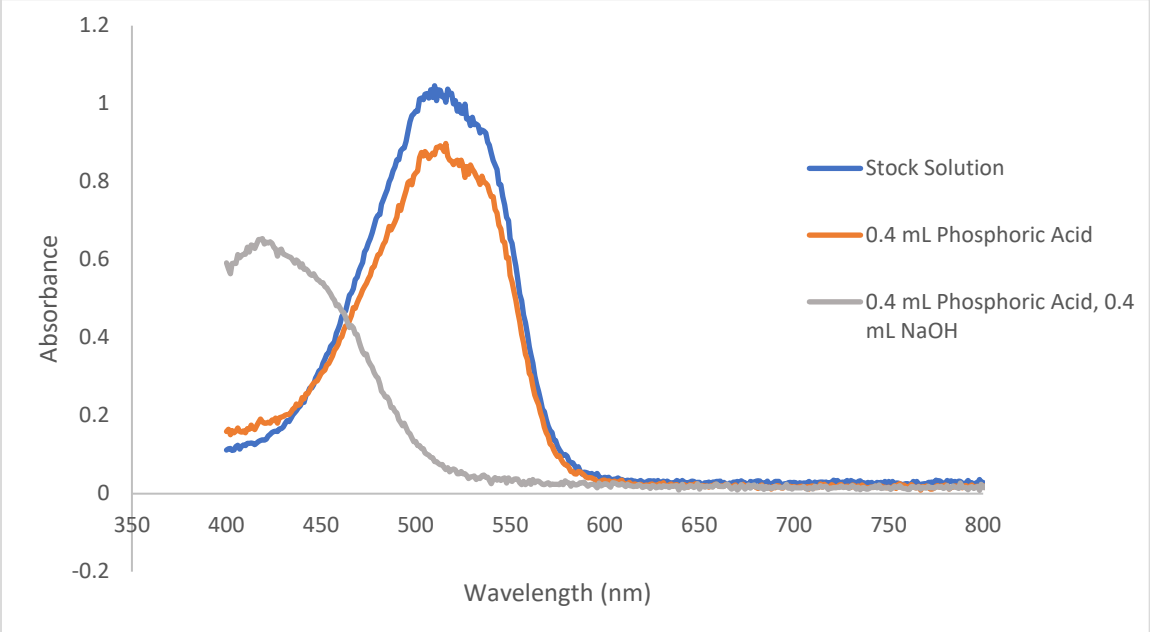


Figure 28. **13** in the presence of phosphoric acid and pH effects of added sodium hydroxide. Addition of anion source did not impact the dye's absorbance, but the addition of base caused a hypsochromic shift.

1.12 UV-Vis studies: a comprehensive conclusion

Two dyes synthesized via separate methodologies, a two-step organic media approach and a one pot route, were analyzed via UV-Vis to assess their potential as ionochromic anion indicators. As visualized via their respective spectra in the presence of different anions at varying concentrations and pH, it was determined that the current dye motif is insufficient to function as a suitable dye. The study was further impeded by the inherent insolubility of certain species, such as tetrabutylammonium chloride, in the solutions of interest. However, it was initially reasoned that these dyes would be able to function as indicators in organic solvents by virtue that species very soluble in aqueous media, such as the anions tested, would more likely be sequestered by the dye molecules. However, this was not observed for any of the anions tested for either dye in any solvent. It was determined that the current azo structure employed is too simplistic and not sufficiently robust to properly "hold" the anions in its core to enable the change in conjugation that allows for detectable changes in absorbance. Therefore, future work could capitalize on this notion of multi-substituted dye structures that allow for a better sequestering by the fact that the anion could become "stuck" within the structure. Figure 29 proposes more complex azo structures, in which the dyes are now multi-substituted.

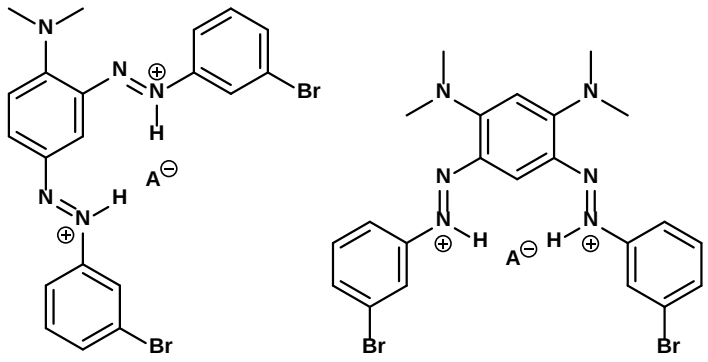


Figure 29. Proposed more complex azo structures with a sequestered anion depicted by A^-.

With this more complex motif, an anion can be more easily captured by the dye molecule's core to negate some of the unfavorable nature imposed by virtue of the anion being in the presence of organic media.

Chapter 2: Synthesis of 1,2-Diphenylethane-1,2-dione

Abstract

A separate project was interested in probing oxidation pathways of 2-Hydroxy-1,2-diphenylethan-1-one (benzoin). Common oxidizing agents include toxic chromium (VI) reagents and harsh, corrosive acids. Sodium nitrate, however, is a more cost-effective reagent and allows for more precise control of stoichiometry resulting from mass measurements of the reagent, as opposed to volume measurements of other oxidizing agents. Thus, the oxidation of benzoin was carried out using sodium nitrate in acetic acid. It was found through melting point, proton NMR, and GC-MS results that the oxidation to 1,2-Diphenylethan-1,2-dione (benzil) was pure and in high yields. The implication of this work is a safer, more cost-effective route of oxidation to be implemented in teaching laboratory experiments.

2.1 The importance of oxidation

Oxidation and reduction reactions—known as redox reactions—are ubiquitous. These types of reactions involve the transfer of electrons between species, where the oxidized species loses electrons and the reduced species correspondingly gains electrons. These species can therefore be described by their oxidation states, which can be positive, negative, or zero. By this definition, species that gain electrons have a decrease in their oxidation state and oxidized species have an increase in their oxidation state. Organic chemical reactions are not as easily defined by these parameters. Typically, when an organic compound is oxidized, this is referred to a carbon center gaining bonds to heteroatoms such as oxygen or sulfur. When a corresponding compound is reduced, this refers to the carbon center gaining bonds to hydrogen and losing bonds to the aforementioned heteroatoms (Figure 30).

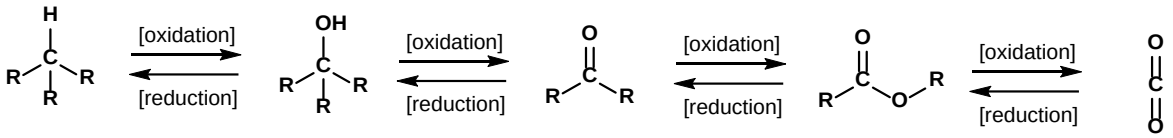


Figure 30. Oxidation and reduction of a carbon center, where O can be replaced with any heteroatom.

Redox reactions compose the basis of life. For instance, photosynthesis involves the process of plants utilizing sunlight to convert this energy into ATP and nicotinamide adenine dinucleotide phosphate (NADPH).[xv] However, redox reactions in biological systems are not wholly beneficial. Oxidative damage and oxidative stress can impose toxic effects on life and mandates a fine balance. In fact, it is the redox potential within a cell that defines this equilibrium. Such oxidative damage can affect proteins, DNA, and lipids. Conversely, reductive stress can also occur if the balance is shifted to a higher reducing power. Therefore, not only is the basis of life redox reactions, but the finely tuned interplay between these processes as well.[xvi]

In organic chemistry, redox reactions yield a pathway to highly functionalized molecules. For instance, oxidizing a primary or secondary alcohol to its corresponding aldehyde or ketone allows for the

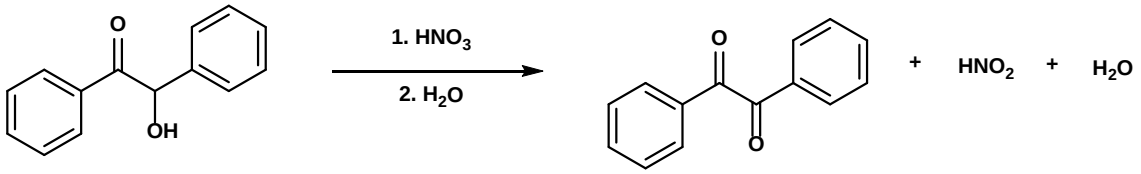
access of new carbon-carbon bond formation through the well-defined Grignard reaction involving the attack of this nucleophile on the electrophilic carbonyl center. Reducing carbonyls or imines to their corresponding alcohol or amine allows for newly available chemistry that their oxidized counterparts could not participate in. Relevant with this oxidation experiment, benzoin was oxidized to benzil in which the product could now participate in an aldol condensation with dibenzyl ketone to yield 2,3,4,5-tetraphenylcyclopentadienone in a later undergraduate laboratory experiment.

2.2 Previously implemented oxidation experiments in the undergraduate laboratory

The alcohol functional group is susceptible to a variety of reactions, including oxidation and reduction transformations. The oxidation of organic molecules involves adding bonds to electronegative atoms, such as nitrogen or oxygen. Different reagents can be utilized to oxidize alcohols, including oxygen, different chromium species (Cr^{6+}), and sodium hypochlorite (common household bleach). Through this oxidation, the alcohol functional group can be transformed into an aldehyde, ketone, or carboxylic acid depending on the nature of the carbon center that is being oxidized and the conditions employed for the oxidation, namely whether or not it is anhydrous. Nitric acid also has the capacity to oxidize this functional group, but this reagent is corrosive and poses dangers upon skin contact and inhalation. Previous experiments implemented in the CHEM 255 course at The Ohio State University utilized an excess of nitric acid (10.5 mL) at reflux in order to oxidize 2-hydroxy-1,2-diphenylethan-1-one (benzoin) to 1,2-diphenylethane-1,2-dione (benzil). Results were mixed, and the isolated product contained a large percentage of unoxidized starting material. Attempting to utilize this product in later experiments, such as for a subsequent aldol condensation, yielded poor results. Therefore, the interest of this project was to find an alternative method of oxidizing benzoin for purer, higher yielding results that also eliminated the use of corrosive reagents in favor of a safer, more cost-effective oxidizing agent.

2.3 Optimizing nitric acid parameters

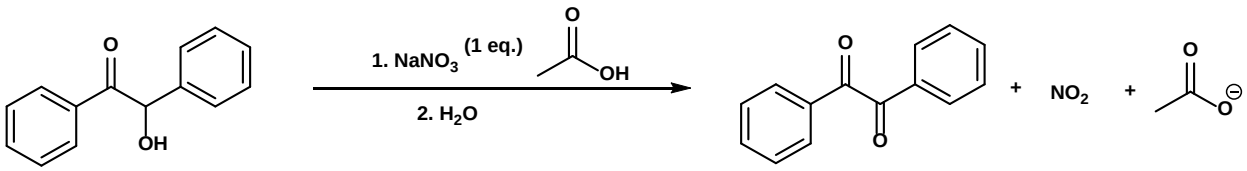
Initially, the intent of the project was to reduce the amount of nitric acid used for safer conditions to be employed in the undergraduate teaching laboratory in addition to reducing waste. The original experiment implemented in CHEM 255 course at The Ohio State University mandated a large excess of nitric acid (Scheme 6). The product obtained following reflux and subsequent recrystallization did not have the characteristic vibrant yellow appearance of benzil.



Scheme 6. Initial oxidation conditions employing excess nitric acid.

During this laboratory sequence, the synthesized benzil was utilized for a later experiment involving the aldol condensation to form 2,3,4,5-tetracyclopentadienone. This synthesized starting material failed to be sufficiently pure to allow for a successful aldol condensation, and so the experiment was discarded from the laboratory sequence. Upon attempting the same oxidation with the excess nitric acid, a similar problem was encountered: an incomplete reaction and the presence of impurities. Therefore, the first step in the process of optimizing conditions for the experiment was to reduce the equivalents of nitric acid. The same parameters as depicted in Scheme 6 were employed for subsequent syntheses, except for the reduction of nitric acid to 7 mL, 4 mL, and then 1 mL. The oxidation massively benefited from the decrease in equivalents; in fact, with the reduction to about 1.1 equivalents of nitric acid, the synthesized product was sufficiently pure and in appreciable yield. Although the issue regarding impure product or an incomplete reaction was eliminated, in addition to massively reducing the acid waste the synthesis produced, the problem of the corrosive and harmful starting material was maintained.

In order to bypass the usage of a reagent as harsh as nitric acid, sodium nitrate was reviewed as a potential candidate to function as an oxidizing agent. The synthesis maintained the same conditions as the nitric acid reflux, except in lieu of utilizing the acid with approximately 1.1 equivalents, this was replaced with sodium nitrate (Scheme 7). Instead of varying the amount of sodium nitrate, the time of reflux was modified to determine optimal conditions to maximize yield without compromising the synthesis for an undergraduate laboratory setting by mandating too much time. The reflux was thus carried out at 30 minutes, 1 hour, and 2.5 hours. Throughout the course of the reflux, red nitrogen dioxide vapors would emit from the condenser insinuating the reaction was still taking place. Therefore, the shorter time periods of 30 minutes and 1 hour were determined to be insufficient timing to allow for the reaction to go to completion since the development of red vapors was still evident after this elapsed time. The red vapors were no longer evident following 2.5 hours of reflux, thereby implying reaction completion and therefore maximized yield. This notion was confirmed following NMR, TLC, and melting point characterization which all showed sufficiently pure benzil in high yields.



Scheme 7. Revised oxidation procedure utilizing 1 equivalent of sodium nitrate.

A cost analysis was also carried out to compare how utilizing either nitric acid or sodium nitrate would impact how economically savvy the potential experiment would be once employed in the undergraduate laboratory setting. The price per student to utilizing nitric acid with Fisher as the vendor was determined to be $0.01. If obtaining sodium nitrate from the same vendor, the cost of reagent would be comparable to that of nitric acid. Sodium nitrate as a reagent was also determined to be more cost-efficient than copper (II) acetate and ammonium nitrate, other potential starting materials that

could replace nitric acid. These reagents, respectively, would cost $0.10 and $0.08 per student. Therefore, replacing nitric acid with sodium nitrate as opposed to copper (II) acetate or ammonium nitrate would not impose additional cost to the undergraduate teaching laboratories. This further substantiated this replacement in addition to concerns regarding waste generated, corrosive starting materials, and questionable purity of synthesized product.

The oxidation experiment is currently employed for Chemistry 2550 utilizing Scheme 7 since the autumn 2016 semester. Since its implementation, the average yield across all students has been 80 ± 10% when accounting from autumn 2016-spring 2018 semesters. In fact, the majority of students isolated 80% yield or better (64% of students), and 98.5% of students isolated 50% yield or better. Therefore, the replacement of nitric acid with sodium nitrate not only allowed for a more cost-efficient synthesis, but also allowed for isolation of an appreciable amount of pure product.

2.4 Potential auto-oxidation of benzoin during GC-MS analysis

Purity of benzil via the oxidation of benzoin experiment was assessed through melting point, GC-MS, TLC, and proton/carbon NMR. Melting point, TLC, and NMR studies verified the purity of the compound, but GC-MS results were initially misleading. In order to assess reaction completion, the synthesized benzil was injected into the instrument in addition to benzoin and benzil supplied by Sigma-Aldrich. The synthesized product matched the identity of the Sigma-Aldrich benzil through direct comparison between the spectra and by comparing to the database. However, the injected benzoin appeared to match the fragmentation patterns presented by benzil. This suggested that the parameters of the GC-MS somehow allowed for the auto-oxidation of benzoin during the injection or elution. Therefore, parameters of the GC-MS including oven temperature and injection temperature were modified to assess how the fragmentation pattern of benzoin might change.

In addition to modifying experimental parameters, additional trials were conducted in an attempt to mimic the possible auto-oxidation outside of the GC-MS. Benzoin was refluxed in isopropyl

alcohol for 10 minutes and then cooled. This was then spotted alongside pure benzoin and pure benzil dissolved in dichloromethane on a TLC plate and eluted with dichloromethane. Results were negative for the oxidation, as evident by the unchanged benzoin spot succeeding the reflux.

A microwave was also utilized in order to mimic GC-MS conditions to assess the possibility of the auto-oxidation of benzoin. To two separate vessels, benzoin was dissolved in water or methanol and ran on the microwave at 150 °C for 15 minutes to replicate the GC-MS default conditions. After allowing the reactions to cool, the possible oxidation was monitored via thin layer chromatography, eluting with dichloromethane, and by also spotting pure benzoin and benzil as references. This also showed no presence of the oxidized product. The water reaction, however, showed streaking on its respective plate and showed the possibility of either trace oxidized product or contamination via water. To analyze more clearly, the reaction was extracted with methanol but yielded no spot when examined via TLC. The same methanol/water samples were heated again in the microwave at 165 °C for 15 minutes; upon analyzing via TLC with dichloromethane as the eluent, the methanol reaction showed the presence of two spots confirmed to be benzil and benzoin when running pure compounds as references. The water reaction again showed no spot, but this could have been a result of solubility: benzoin and benzil are nearly insoluble in water.

Simultaneously, additional tests on the GC-MS were carried out in different solvents to analyze oxidation conditions of benzoin. Injecting benzoin in 200-proof ethanol and in 1:1 acetone:dichloromethane utilizing default GC-MS parameters both showed a 87% match with benzil according to the database, which suggests that oxidation could be occurring in the instrument. This affirmed what was previously observed in two prior injections of benzoin in 200-proof ethanol. However, these results also contested conclusions found previously: a previous injection of benzoin in the 1:1 solution using the same parameters did not show an oxidation. Through the testing of different parameters for benzoin and benzil in differing solvents and instrumental methods, it was determined

that the probability of auto-oxidation was low. It was predicted that both benzil and benzoin fragment in similar patterns compared to each other, which supports why the observed spectrum for benzoin showed similar matches to the database for benzil (Figure 31). The auto-oxidation experiments were not relevant in regard to the implementation to the undergraduate teaching laboratories.

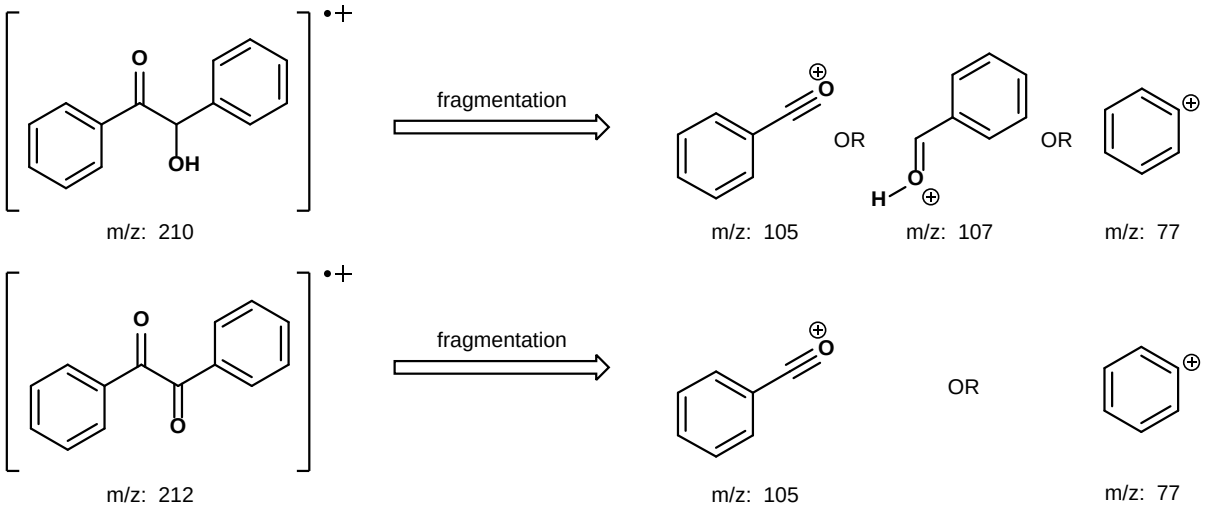


Figure 31. Potential fragmentation patterns for benzoin and benzil. Both feature similar fragmentation patterns, which potentially refutes auto-oxidation.

2.5 Conclusion

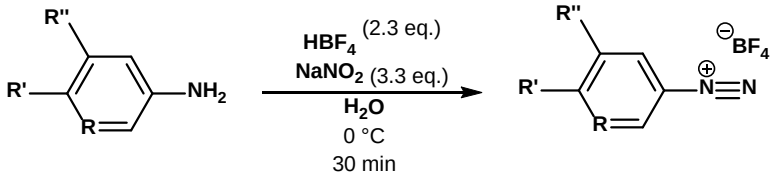
The oxidation of benzoin initially implemented in the undergraduate teaching laboratory utilized an excess of nitric acid at reflux which resulted in poor yield in conjunction with impure product. To mitigate this issue, nitric acid was gradually reduced to 1:1 stoichiometrically. However, this reagent is corrosive and a more dangerous alternative to the updated starting material, sodium nitrate. Utilizing one equivalent of this oxidizing agent yields pure product in high yields without imposing the same safety issues as nitric acid. Additionally, cost analysis showed that sodium nitrate was just as financially feasible to implement into the undergraduate laboratory as nitric acid.

Experimental Methods

Synthesis of 2-hydroxy-3-((3-nitrophenyl)diazenyl)naphthalene-1,4-dione (compound 14): General Procedure 1 -aqueous diazotization and azo coupling. A suspension of 3-nitroaniline (0.536 g, 3.88 mmol), water (1.20 mL), and hydrochloric acid (9.6 mmol, 1.60 mL, 6 M) was prepared at 0 °C. A separate solution of sodium nitrate (0.414 g, 4.87 mol) in water (0.80 mL) was prepared at 0° C. The nitrate solution was added dropwise to the aniline suspension over 15 min and stirred for an addition 10 min after the addition was completed. To this diazonium solution, small portions of sodium was added until the reaction was neutral (pH = 7). A suspension of 2-hydroxy-1,4-naphthoquinone (0.673 g, 3.86 mmol), sodium bicarbonate (0.326 g, 3.88 mmol), and water (5.50 mL) was prepared and immediately added to the neutralized solution and the reaction was allowed to stir for 1.5 h. Then, hydrochloric acid (10 mmol, 10 mL, 1 M) was added to acidify the reaction (pH = 3). The solid was isolated via vacuum filtration, washing with water (1 mL) to yield an intense orange/red powder (2.26 g, 6.99 mmol, 97%).

Synthesis of (E)-4-((3-bromophenyl)diazenyl)-N,N-dimethylaniline (compound 13): General Procedure 2 - diazotization and azo coupling in acetonitrile. A solution of N,N-dimethylaniline (0.019 g, 0.158 mmol, 0.02 mL) in acetonitrile (4 mL) was prepared at 0° C. A separate suspension of synthesized 3-bromobenzenediazonium tetrafluoroborate (0.055 g, 0.287 mmol) in acetonitrile (4 mL) was prepared at 0 °C. This benzenediazonium suspension was added dropwise to the aniline solution over 26 min with stirring. The reaction was stirred for an additional 15 min after complete addition at 0° C and then evaporated under reduced pressure at 35 °C for 15 min to yield a red wine solid (0.0586 g, 0.193 mmol, 117%).

Synthesis of aryldiazonium tetrafluoroborate salts (General Procedure 3). A solution of an aniline (0.1-0.8 mmol) was prepared in tetrafluoroboric acid (8.42 mmol, 1.1 mL) at 0 °C. A separate solution of sodium nitrite (x mmol) in water was prepared at 0 °C, and then was added to the aniline solution slowly and dropwise over a period of 10-15 min with stirring. After stirring for an additional 10 min at 0 °C, the salt was isolated via vacuum filtration, washing with tetrafluoroboric acid (3.5 mL), 190-proof ethanol (2 x 3.5 mL), and diethyl ether (3 x 3.5 mL) to yield a powdery solid. Used without further characterization owning to the unstable nature of the diazonium species.

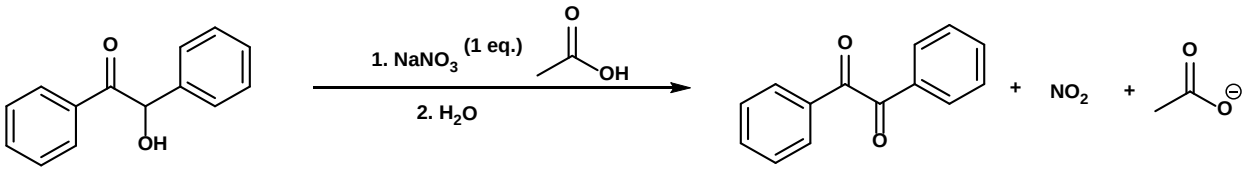


substituted aniline

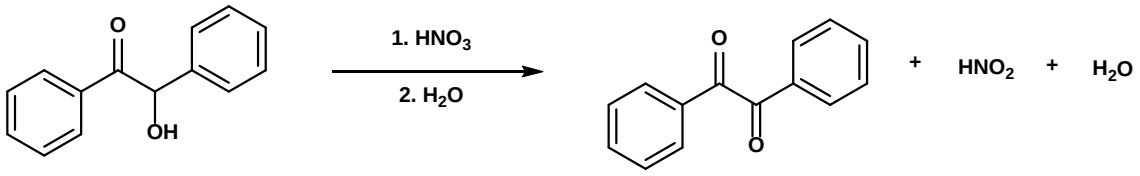
diazonium

2: R=CH, R'=H, R''=F
3: R=CH, R'=OH, R''=H
4: R=CH, R'=H, R''=COOH
5: R=CH, R'=H, R''=Br
6: R=CH, R'=H, R''=NO_2
7: R=N, R'=H, R''=H
8: R=CH, R'=H, R''=Cl
9: R=CH, R'=CN, R''=H
10: R=CH, R'=H, R''=SO_3H
11: R=CH, R'=CF_3 R'=H
12: R=CCOOH, R'=H, R''=COOH

Synthesis of 1,2-diphenylethane-1,2-dione (sodium nitrate variant). A suspension of benzoin (1.50 g, 7.07 mmol), sodium nitrate (0.601 g, 7.07 mmol), and acetic acid (7.25 mL) was prepared at 0 °C and refluxed for 2.5 h. The reaction was then poured over crushed ice and the crude product was isolated via vacuum filtration. The crude product recrystallized from 200-proof ethanol, using water to achieve the cloud point, to obtain a yellow, needle-like solid.



Synthesis of 1,2-diphenylethane-1,2-dione (nitric acid variant). A suspension of benzoin (1.50 g, 7.07 mmol), acetic acid (7.25 mL) was prepared at 0 °C. Then, nitric acid (10 mL, 4 mL, 1 mL) was added to this suspension slowly at 0 °C. The reaction was refluxed for 2 h and subsequently quenched by pouring over crushed ice. The crude product was isolated via vacuum filtration and then recrystallized from 200-proof ethanol, using water to achieve the cloud point, to obtain a yellow, needle-like solid.



UV-Vis Studies

First, each dye was tested in a variety of solvents to appraise solubility. It was determined that SAM-2-156 had appreciable solubility in chloroform, and SAM-3-9 was soluble in acetone, acetonitrile, and chloroform. Therefore, UV-Vis studies were warranted for these aforementioned solvents. Neither dye was soluble in water, regardless of pH. To prepare the initial stock solution, first a solution of 10 mM of methane sulfonic acid in the desired solvent was prepared in a 250-mL volumetric flask. A background absorbance spectrum was obtained. To a 100-mL volumetric flask, 100 mL of the initial stock solution was added. Then, the dye stock solution was prepared by adding the dye in small increments to the 100-mL volumetric flask until an absorbance of 1 was obtained at the maximum wavelength absorption. A spectrum of this dye solution, without the presence of anions, was obtained. Utilizing the same initial stock solution, 1 M testing solutions of hydrochloric acid, hydrobromic acid, phosphoric acid, tetrabutylammonium bromide, and nitric acid were prepared in 10-mL volumetric flasks. The dye stock solution was partitioned into 10 mL testing volumes for each testing solution. Testing solutions were added in 0.1 mL increments to its respective dye stock solution until a total of 0.4 mL of the testing solution was added. After each addition, a UV-Vis spectrum was obtained. To assess for a pH

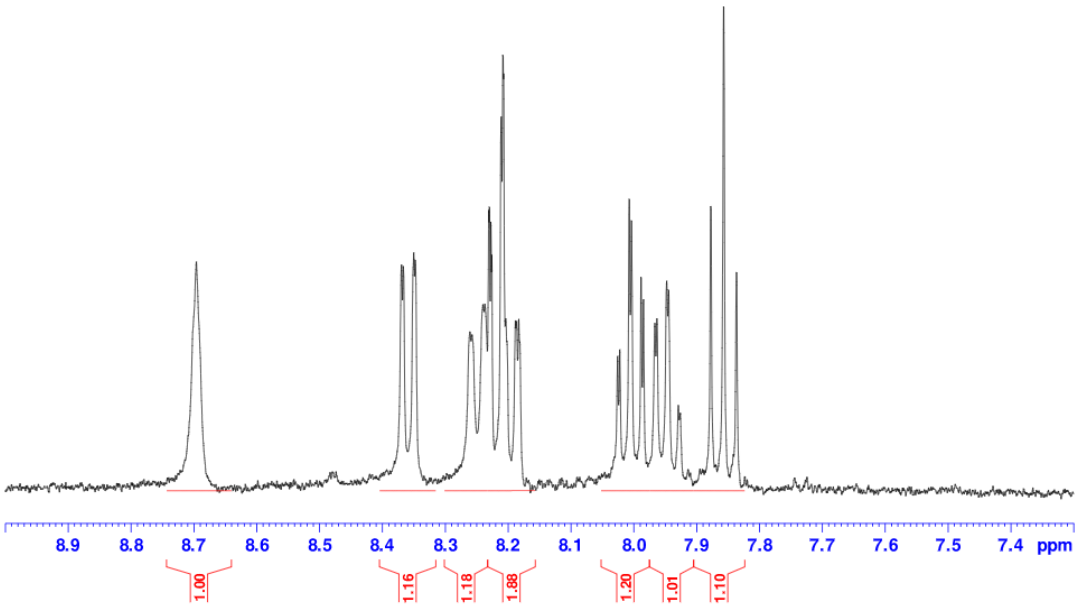
independent effect, dropwise increments of 0.5 M NaOH was added to the dye/anion solution and a UV-Vis spectrum was obtained after each addition to monitor behavior.

UV-Vis Experimental Parameters

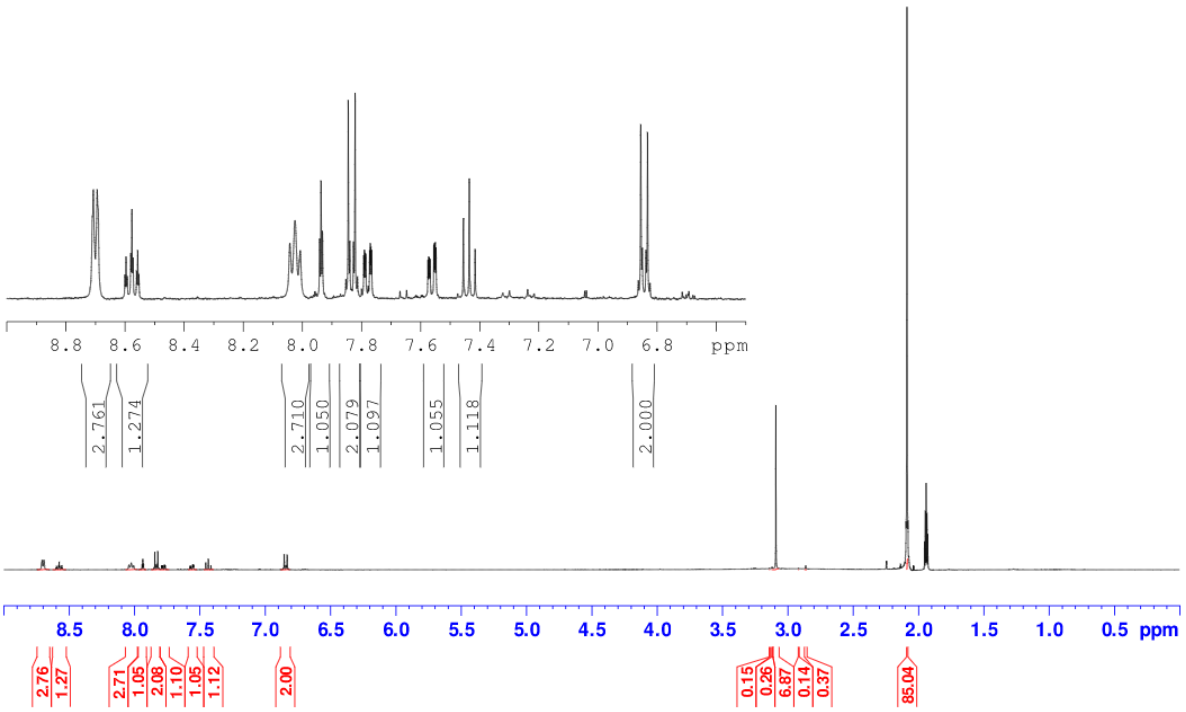
All UV-Vis data was taken on the Ocean Optics USB-650-VIS/NIR scanning from X to X nm with an integration time of 27 ms.

Appendix

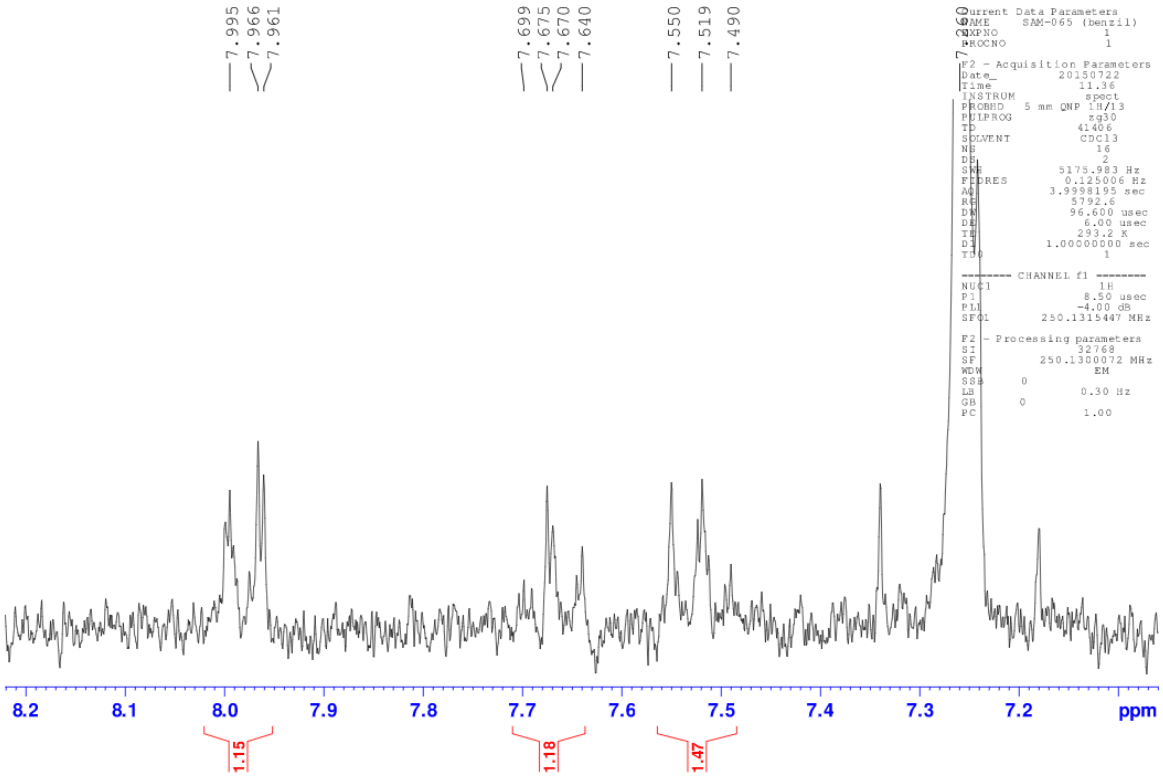
1. NMR spectrum for **14** in acetone-d_6



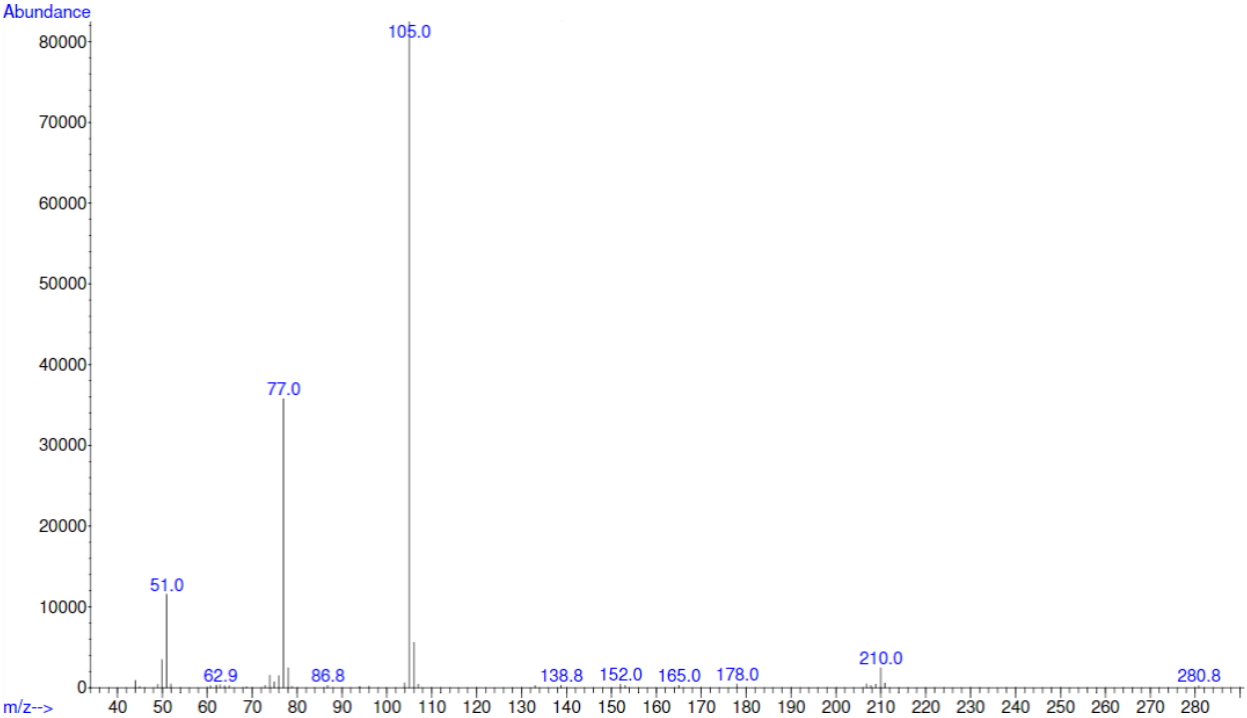
2. NMR spectrum for **13** in acetonitrile-d_3



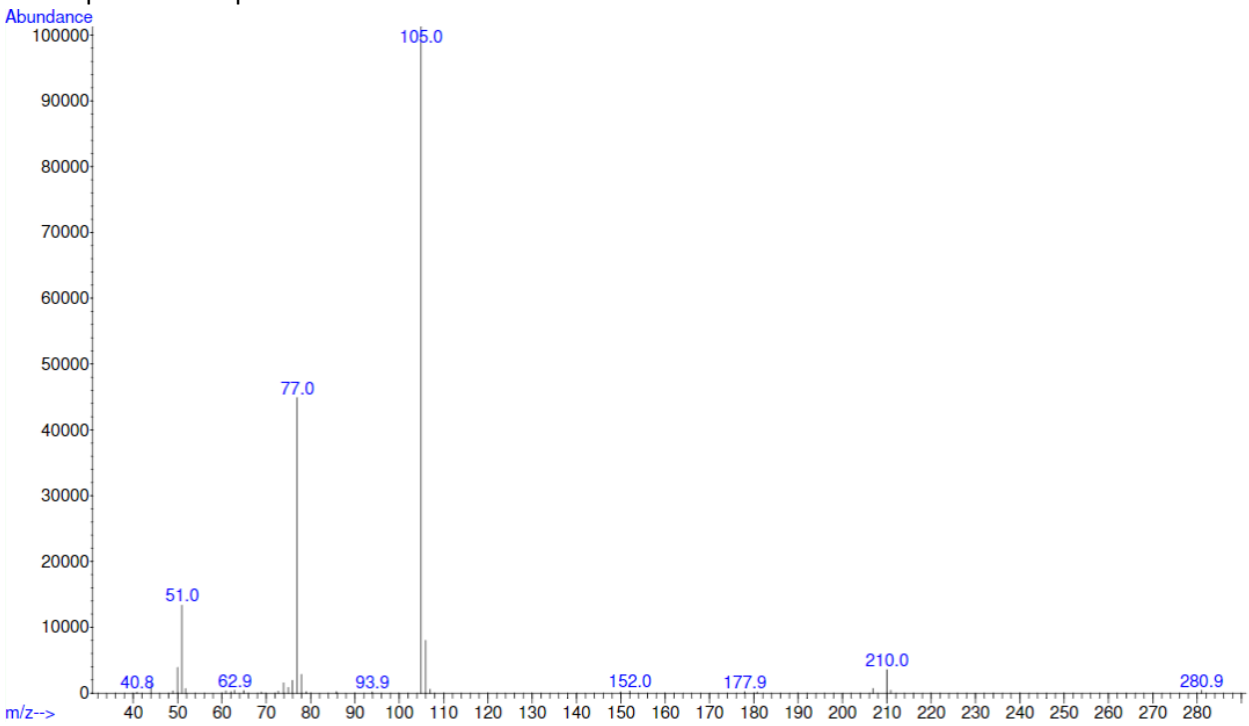
3. NMR of benzil in chloroform-d_3, zoomed in on aromatic region



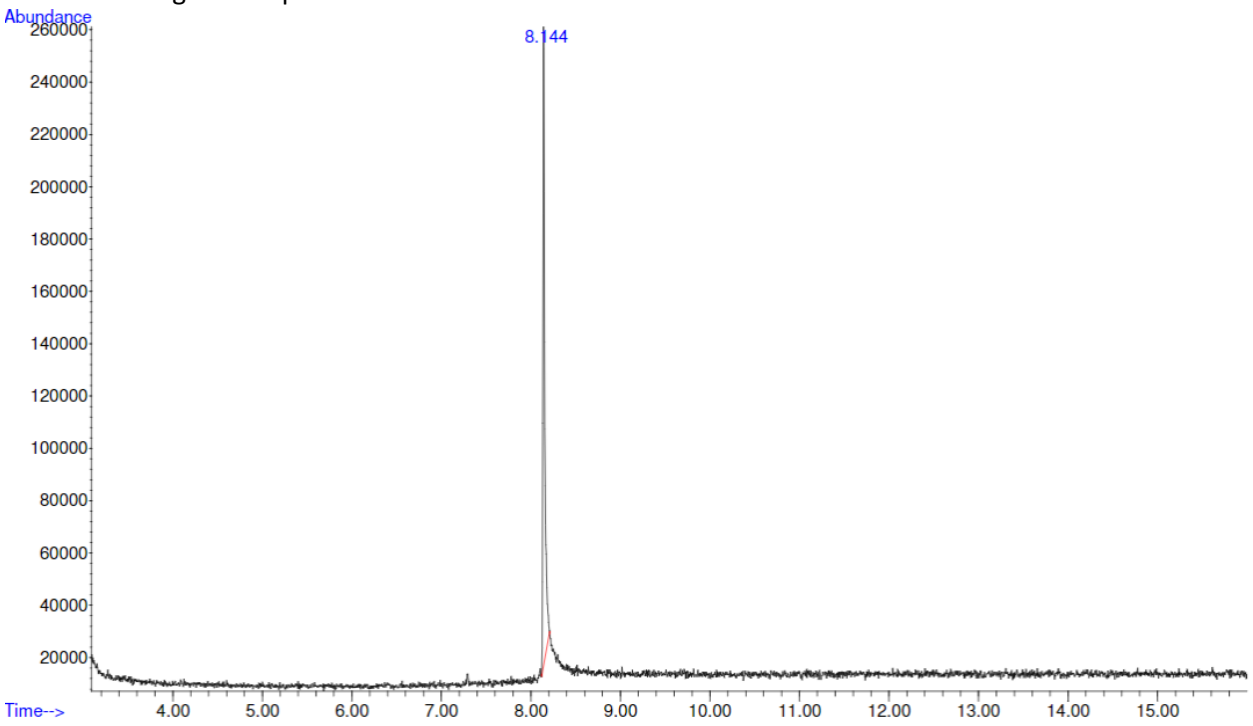
4. Mass spectrum for pure benzoin in ethanol



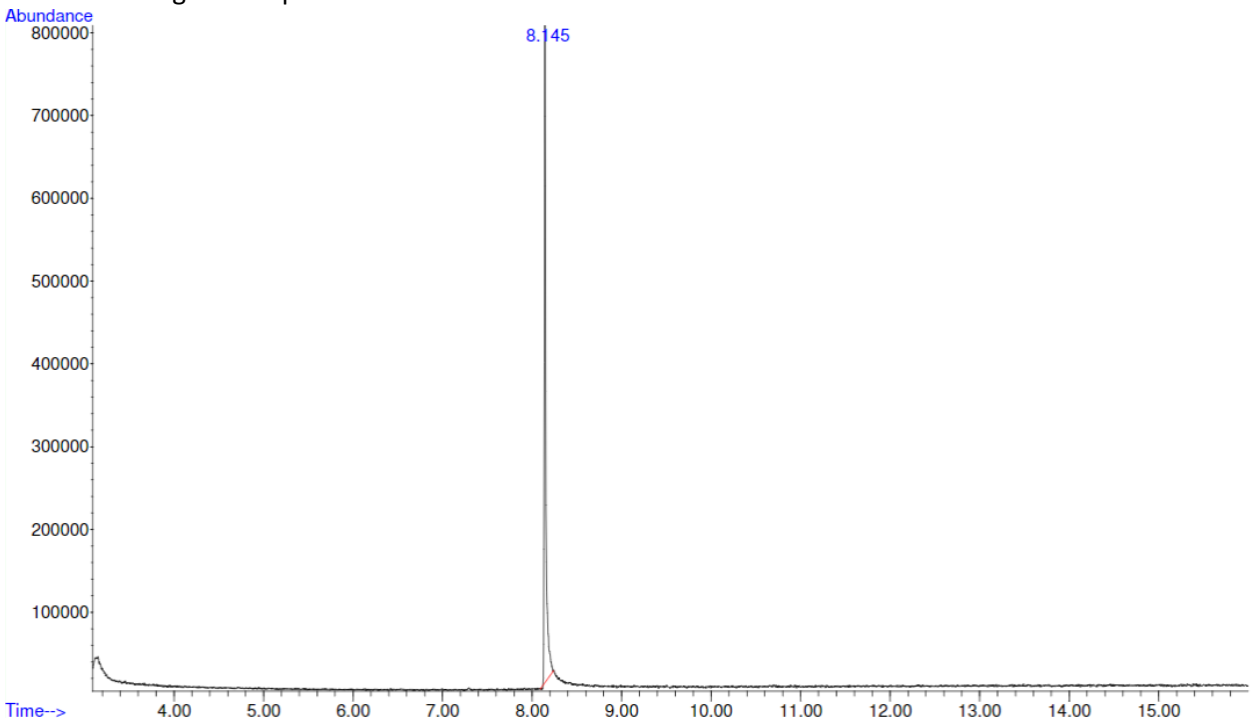
5. Mass spectrum for pure benzil in ethanol



6. Gas chromatogram for pure benzoin in ethanol



7. Gas chromatogram for pure benzil in ethanol



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